Department of Molecular Medicine,
National Public Health Institute, Helsinki, Finland,
and Department of Medical Genetics,
University of Helsinki, Helsinki, Finland

THE SEARCH FOR GENES PREDISPOSING TO OBESITY

Miina Öhman

Academic Dissertation

To be publicly discussed with the permission of
The Medical Faculty of the University of Helsinki,
In the Auditorium 2 of the Meilahti Hospital,
Haartmaninkatu 4, Helsinki,
On March 30th, 2001, at 12 noon

Helsinki 2001

Supervised by

Prof. Leena Peltonen (Palotie)
Department of Molecular Medicine
National Public Health Institute, and
Department of Medical Genetics
University of Helsinki,
Helsinki, Finland

Reviewed by

Prof. Markku Savolainen
Department of Internal Medicine
University of Oulu,
Oulu, Finland

David B. Allison, Ph.D.
Obesity Research Center, St. Luke's/Roosevelt Hospital,
Institute of Human Nutrition,
Columbia University College of Physicians & Surgeons,
New York, New York, USA

Publications of the National Public Health Institute
KTL A3/2001

Julkaisija – Utgivare – Publisher

Kansanterveyslaitos (KTL)
Mannerheimintie 166
FIN-00300 Helsinki
puh. vaihde 09-47441, telefax 09-4744 8480, URL: www.ktl.fi

Folkhälsoinstitutet
Mannerheimvägen 166
FIN-00300 Helsingfors
tel. växel 09-47441, telefax 09-4744 8480, URL: www.ktl.fi

National Public Health Institute (NPHI)
Mannerheimintie 166
FIN-00300 Helsinki
phone +358-9-47441, telefax +358-9-4744 8480, URL: www.ktl.fi

ISBN 951-740-205-8
ISSN 0359-3584
ISBN 951-45-9878-4 (PDF version) ethesis available at http://ethesis.helsinki.fi/

Yliopistopaino
Helsinki 2001

Everything takes longer than you think.
William Shakespeare 1564-1616

To my dear family

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals. In addition some unpublished data are presented.

I Oksanen L, **Öhman M**, Heiman M, Kainulainen K, Kaprio J, Mustajoki P, Koivisto V, Koskenvuo M, Jänne OA, Peltonen L, Kontula K: Markers for the gene ob and serum leptin levels in human morbid obesity. *Human Genetics 99: 559-564, 1997*

II **Öhman M**, Oksanen L, Kainulainen K, Jänne OA, Kaprio J, Koskenvuo M, Mustajoki P, Kontula K, Peltonen L: Testing of human homologues of murine obesity genes as candidate regions in Finnish obese sib pairs. *European Journal of Human Genetics 7(2):117-124, 1999*

III **Öhman M**, Oksanen L, Kaprio J, Koskenvuo M, Mustajoki P, Rissanen A, Salmi J, Kontula K, Peltonen L: Genome-wide scan of obesity in Finnish sibpairs reveals linkage to chromosome Xq24. *Journal of Clinical Endocrinology & Metabolism 85(9):3183-3190, 2000 Sep*.

IV Perola M*, **Öhman M***, Hiekkalinna T, Leppävuori J, Pajukanta P, Wessman M, Lange K, Koskenvuo M, Palotie A, Kaprio J, Peltonen L: QTL analysis of body-mass index and stature by combined analysis of five Finnish genome scans. *Submitted for publication*

*These authors contributed equally to the respective work.

Publication I also appears in the thesis of Dr. Laura Oksanen (4/1999).

ABBREVIATIONS

AGRP	agouti-related peptide
ASP	agouti-signaling protein
BMI	body-mass index
BMR	basal metabolic rate
bp	base pair
CART	cocaine- and amphetamine regulated transcript
cM	centimorgan
CPE	carboxypeptidase E
DNA	deoxyribonucleic acid
DZ	dizygotic
GDB	Genome Database
h^2	heritability
5HT2CR	serotonin receptor-2C
IBD	identical by descent
IBS	identical by state
LD	linkage disequilibrium
LEP	leptin
LEPR	leptin receptor
Lod	logarithm of odds
MC3R	melanocortin-3 receptor
MC4R	melanocortin-4 receptor
MLS	maximum likelihood score
mRNA	messenger ribonucleic acid
MSH	melanocyte stimulating hormone
MZ	monozygotic
n	number
NIDDM	non-insulin dependent diabetes mellitus
NPY	neuropeptide Y
PC1	prohormone convertase-1
PCR	polymerase chain reaction
POMC	pro-opiomelanocortin
QTL	quantitative trait locus
r	ratio
RR	relative risk
SNP	single nucleotide polymorphism
UCP	uncoupling protein
VC	variance components
WHO	World Health Organization
WHR	waist-to-hip ratio

ABSTRACT

Obesity is an increasing health problem all over the world and the prevalence of overweight increases in every age and social class. Obesity is a multifactorial trait, both genetic and environmental factors contributing to its development. Although the combined action of susceptibility genes and environmental risk factors cannot be easily separated, it has been estimated that 40-80% of the variation in body weight is due to genetic factors. In recent years, the field of obesity research has focused strongly on identifying genetic determinants behind rodent and human obesity. To date, well over 200 chromosomal regions have been implicated in obesity or its related traits in different populations.

This study was carried out to elucidate genetic factors influencing the complex phenotype of obesity in the Finnish population. The original study sample set included 100 obese sibling pairs ascertained from the Finnish Twin Cohort and the weight-reducing groups of Helsinki University Central Hospital. Additional sib pairs were collected later from other hospitals' weight reducing groups. All the probands and their siblings were markedly obese having a body-mass index (BMI) equal to or over 32 kg/m^2.

Firstly, the newly discovered obesity gene, leptin (*ob*) was studied. An association was found between the leptin gene locus and serum leptin levels but no evidence of linkage or association to obesity could be found. Secondly, several biologically relevant candidate genes implicated in rodent obesity, were tested for linkage to human obesity. From seven analyzed gene regions, evidence of linkage to obesity was found on chromosome 18q21.3 which contains the melanocortin -4 receptor gene. Further, dissection of the study material into subgroups to homogenize the phenotype strengthened the linkage yielding a p-value of 0.001. Thirdly, a three-stage genome-wide scan was carried out to identify novel genetic loci predisposing to obesity in the Finnish population. Two major findings were a linkage to chromosome Xq24 (maximal lod score of 3.5) and a linkage to the melanocortin –4 receptor gene. Interestingly, a positional candidate gene on Xq24 region, the serotonin receptor -2C, has been implicated in murine and human obesity.

Finally, as a proof-of-principle study, a quantitative trait locus (QTL) analysis of body-mass index and body height was carried out by combining genotype data from five Finnish genome scans of different complex traits. With a variance component method no linkage to BMI could be detected, but the body height revealed strong evidence of linkage to chromosomes 18q (maximum lod score of 3.0), 7pter (maximum lod score of 2.8) and 12p (maximum lod score of 2.26).

INTRODUCTION

There are some common beliefs on the origin of obesity, such as "Obesity is the consequence of gluttony and sloth". Also, people have observed that obesity runs in families, therefore "it must be in the genes". Both aspects are partially correct. Obesity is a complex disease resulting from a combination of genetic susceptibility, increased availability of high-energy foods and decreased requirement for physical activity in modern society. Obesity has reached epidemic proportions in industrialized countries, and because it is associated with a significant increase in morbidity and mortality (Kopelman 2000), the health care costs directly attributable to obesity have dramatically increased over the past few decades (Kuczmarski et al. 1994; WHO 1997). In Finland, the direct costs for obesity in 1997 were approximately 2.1 billion Finnish marks, or approximately 4.2 % of the total health care expenditures (Pekurinen et al. 2000). In addition to the adult population, also the prevalence of obesity in young children and adolescents has increased (Livingstone 2000). This rapidly expanding problem and the increasing public health impact have strongly promoted research into the aetiopathogenesis of the complex phenotype of obesity.

The first law of thermodynamics, which states that the amount of stored energy equals the difference between energy intake and work, is uniformly applicable to biologic systems. The primary form in which potential chemical energy is stored in the body is fat, i.e. triglycerides, due to its hydrophobic nature and high caloric density that permit extremely efficient way of storage without adverse osmotic consequences (Rosenbaum et al. 1997). Since the adipose mass tends to be relatively stable over long periods in most mammals, it was suggested in the 1950s that a homeostatic mechanism monitors changes in energy stores and elicits compensatory changes in food intake and energy expenditure to maintain adipose mass at a set point (Kennedy 1953). However, this homeostatic mechanism tends to function much more efficiently in the "positive" direction, i.e. the set point is easily exceeded.

In other words, although the human body has excellent physiological mechanisms to defend against body weight loss, it has only weak physiological mechanisms to defend against body weight gain when food is abundant. This is related to the hypothesis of the "thrifty genotype" (Neel 1999) that was proposed several decades ago from studies of diabetes. It argues that living on the edge of survival for a sufficiently long period had fixed in the gene pool a collection of alleles that promote storage of fat in times of plenty. To be conserved in the gene pool, such alleles would have to promote survival in times of famine to a greater extent than they might imperil health and reproductive efficiency, even if, as proposed originally, they were to promote type II diabetes directly. Until a few years ago, the nature of these "thrifty genes" was uncertain, but family studies and clinical analyses have finally provided encouraging evidence of genes that contribute to human obesity by altering the efficiency of energy storage in adipocytes without compromising the survival or reproductive efficiency (Cone 2000).

Obesity is a multifactorial and polygenic trait, and the estimates of its genetic component, i.e. the heritability, can only be population and time specific. The predisposing genes and alleles of each individual interact in various ways with different environments, and thus, it is not evident to predict, how and of which components the final phenotype was evolved. A susceptibility gene increases the risk for the disease, but is not absolutely necessary for the expression of the disease: it only lowers the threshold

for the development of the particular disease. To date, molecular genetic studies have identified putative loci affecting obesity-related phenotypes in all autosomes and in chromosome X. The number of genes, markers and chromosomal regions that have been linked or associated with the human obesity phenotypes is now well above 200 (Chagnon et al. 2000). Specifically, there are about 100 reports of positive associations pertaining to 40 candidate genes, 44 loci have been linked to obesity or its indicators in genome scans and other linkage studies. There are over 100 quantitative trait loci contributing to obesity identified in different animal models. Twenty Mendelian disorders that have obesity as one of the minor clinical features, have been assigned to a chromosomal location. Finally, rare human cases of pathological obesity can now be explained by a mutation in a single gene (Chagnon et al. 2000).

It is important to investigate the molecular mechanisms underlying weight regulation and feeding, since the results of basic research can be used to develop pharmaceutical agents to reduce weight. By reducing obesity in society, the health care costs of associated diseases will presumably also be decreased. However, the ultimate goal of investigators, the development of medicines starting from the cloning of the disease gene, will take several years even with the newest technology. In view of this, as far as the effectiveness in public health care of common diseases is concerned, it may be more important to control for potential environmental risk factors rather than genetic determinants because they are more easily influenced in most cases.

REVIEW OF THE LITERATURE

1. OBESITY: DEFINITION, CAUSES AND CONSEQUENCES

1.1. Definition and measures of obesity

Obesity has been defined by some as a preventable disease in which as excess amount of fat tissue results from an imbalance between energy intake and energy expenditure (WHO 1997; WHO 1995). Obesity can be measured by several methods, but the most widely used and readily available for research purposes is the body-mass index (BMI), which is easily derived from both objectively measured as well as self-reported data, and is calculated by dividing weight in kilograms by height in meters squared (kg/m^2). This method was originally developed to eliminate the influence of body height on weight, i.e. to obtain a measure for *relative* body weight. Investigations have been made to determine weight-height index (wt/ht^n) would be the most suitable for eliminating the effect of height, and which index would be the most specific for different ages and populations (Goldbourt and Medalie 1974; Khosla and Lowe 1967). For example, the ponderal index (kg/m^3) is the most suitable weight-height index for newborns. The adequacy of a certain weight-height index depends also on the selected population, but on average, the body-mass index (kg/m^2) measures relative weight sufficiently well. Thus, it is widely used internationally and allows comparisons between studies from different populations.

The body-mass index correlates with body fatness (Gray and Fujioka 1991; Rosenbaum and Leibel 1988; Strain and Zumoff 1992) and therefore, it is widely used in obesity studies. However, because height affects the value of BMI non-linearly, in very tall or very small persons the BMI gives a distorted measure of body fatness. In very tall individuals, the increase in body fat (excess weight) does not increase the value of BMI in the same proportion as it does in very small individuals: the increase in BMI equals to the change of weight (kg) multiplied by $1/(height)^2$. Furthermore, BMI depends on sex and age: in women and in older people, fat tissue usually consists a greater portion of total body mass (Gallagher et al. 1996). Compared with other measures of obesity, BMI correlates significantly with the waist-to-hip ratio ($r=0.52$) but less markedly with skinfold (subscapular/triceps ratio) measurements ($r=0.18$) (Cardon et al. 1994). To only measure the proportion of body fat, the underwater-weighting or bioimpedance are more accurate, and magnetic resonance imaging (MRI) can be used to determine virtually the exact amount and distribution of fat in each compartment of the body. Yet, these methods require considerably more resources and thus, may not be available for every study.

BMI cut-off points for definition of obesity were introduced by the World Health Organization in 1995 and 1997 (WHO 1997; WHO 1995) (Table 1).

Table 1. The body-mass index (BMI) cut-off points proposed by the World Health Organization to define obesity by different classes.

Body-mass index (kg/m^2)	Weight class
BMI < 20	underweight
20< BMI <25	normal weight
25< BMI <27	slight overweight
27< BMI <30	moderate overweight
30< BMI <35	obesity
35< BMI <40	severe obesity
BMI > 40	morbid obesity

1.2. Prevalence of obesity

The prevalence of obesity is increasing globally and also in undeveloped countries due to the westernization of lifestyles (Grol et al. 1997; Hodge et al. 1996). According to the WHO classification (Table 1), 54% of adults in the United States are overweight (BMI ≥ 25 kg/m^2) and 22% are obese (BMI ≥ 30 kg/m^2) (Flegal et al. 1998). The prevalence trends of weight gain and obesity in European countries are similar to those in the U.S. (Seidell 1995a) although specific rates are lower.

Compared to European countries, the prevalence of obesity in Finland is high, and it is highest among the Scandinavian countries (Pietinen et al. 1996). The average BMI is higher in Finnish men and women than in their Swedish counterparts (Rahkonen et al. 1998). In 1992, the prevalence of overweight (BMI ≥ 25 kg/m^2) in Finnish men was 63% and in women 49%, and the prevalence of obesity (BMI ≥ 30 kg/m^2) was 19% in men and 18% in women. Despite the accentuated rise of being overweight and obesity in Finland during the past 30 years (Lahti-Koski et al. 2000), severe and morbid obesity have remained rare, at least when compared to the United States where 8% of the population is severely obese (Laurier et al. 1992; Pietinen et al. 1996). Only 3.0% of Finnish men and 5.5% of women were severely obese (BMI ≥ 35 kg/m^2) and 0.5% of men and 2.1% of women were morbidly obese (BMI ≥ 40 kg/m^2) in 1992 (Pietinen et al. 1996). These rates might be now higher according to a more recent study (Rahkonen et al. 1998).

1.3. Aetiology and risk factors for obesity

Body weight is determined by an interaction between genetic and environmental, e.g. psychosocial, factors acting through the mediators of energy intake and energy expenditure. Although genetic predisposition is important in gaining weight, the marked rise in the prevalence of obesity during the past two or three decades is best explained by

behavioral and environmental changes (Hill and Peters 1998). Our genes are unlikely to have changed substantially after the Second World War, but the availability of highly palatable, energy-dense foods and an environment that discourages to physical activity are more prevalent than ever.

A sedentary lifestyle with decreased physical exercise promotes obesity (Esparza et al. 2000; Heitmann et al. 1997; Williamson et al. 1993) in adults, children and adolescents (Davies et al. 1995; Maffeis 2000). Of dietary factors that have been associated with weight gain and obesity, the most important are high fat content (Bray and Popkin 1998), energy density, pleasantness of taste (i.e. palatability) and dietary variety (McCrory et al. 2000). With respect to behavioral aspects, overweight subjects have been reported to have more difficulties in controlling eating, stronger feeling of hunger (Hakala et al. 1999), a tendency to engage in emotional eating (Lindroos et al. 1997) and binge eating (Wilson et al. 1993). Socioeconomic factors such as low levels of education and income, and social isolation are associated with weight gain and predictive of the development of obesity (Kahn and Williamson 1990; Kuczmarski et al. 1994; Pietinen et al. 1996; Rahkonen et al. 1998; Rissanen et al. 1991).

1.4. Morbidity and mortality associated with obesity

Obesity causes, exacerbates and predisposes to many diseases, e.g. osteoarthritis, cancer of the breast and colon, hypertension, gallbladder disease (Colditz 1999) and in particular to coronary heart disease (Hubert et al. 1983; Manson et al. 1990) and to non-insulin-dependent diabetes mellitus (NIDDM) (Colditz et al. 1995; Olefsky and Kolterman 1981). Obesity ranks among the major determinants of health care costs in westernized countries (Seidell 1995b). According to different estimations the direct health care costs attributable to obesity vary between 2-7 % of the total health care expenditures in several countries including Finland (Levy et al. 1995; Pekurinen et al. 2000; Seidell 1995b; WHO 1997). In 1997 the direct health care costs of obesity in Finland were estimated to be 0.9-3.2 billion Finnish marks, and thus obesity represents even a greater burden to health care than smoking (Pekurinen et al. 2000). In the United States the direct costs of inactivity and obesity account for some 9.4 % of the national health care expenditures, and in 1995 the direct costs for obesity (BMI > 30 kg/m^2) were app. 70 billion dollars (Colditz 1999).

Some negative influences of obesity are, to some extent, dependent on the regional distribution of the adipose tissue. Intra-abdominal visceral fat is a major contributor to the development of hypertension, elevated plasma insulin concentration and insulin resistance, NIDDM and hyperlipidaemia (Kopelman 2000). Increased body fat leads to worsened cardiovascular functions, by increasing total blood volume and cardiac output, and thus causing structural changes in the heart, e.g. left ventricular dilatation and myocardial hypertrophy (Lauer et al. 1991). Independent of several traditional risk factors, body weight is directly associated with the development of congestive heart failure (Hubert et al. 1983).

The association between overweight and the risk of type II diabetes is linear. In a prospective population study, in women the risk of diabetes increased fivefold for those with a BMI of 25 kg/m^2, 28-fold for those with BMI of 30 kg/m^2 and 93-fold for those with BMI ≥ 35 kg/m^2, compared to controls with BMI ≤ 21 kg/m^2 (Colditz et al. 1990). In men, the risk of diabetes is increased 2.2-fold when 25 ≤ BMI < 27 kg/m^2, 6.7-fold

when 29 ≤ BMI < 30 kg/m^2, and 42-fold for those with BMI ≥ 35 kg/m^2 (Chan et al. 1994). Obesity also increases the risk of obstructive sleep apnoea (Kopelman 1992; Palomaki et al. 1992), certain forms of cancers (den Tonkelaar et al. 1995) and osteoarthritis of weight bearing joints (Kopelman 2000).

Obesity is associated with increased mortality in both sexes in several studies (Manson et al. 1995; Troiano et al. 1996). Throughout the range of moderate to severe obesity, the risk of death due to all causes, e.g. cardiovascular disease and cancer, is increased (Calle et al. 1999). The risk rises remarkably when the BMI exceeds 32 kg/m^2, and when the BMI exceeds 42 kg/m^2, the risk of death is 2.6-fold in men and 2.0-fold in women compared with normal weight controls (Calle et al. 1999). The association between BMI and mortality is suggested to be U-shaped, and as an explanation to this, the mortality related to low BMI could be attributed to confounding factors such as smoking, pre-existing or chronic diseases (Losonczy et al. 1995). However, Allison et al. showed by meta-analysis that pre-existing disease does not confound the BMI-mortality association, or eliminating early deaths is inefficient for reducing that confounding (Allison et al. 1999a). Yet, the risk of dying is a linear increasing function of high fat mass and low fat-free mass (Heitmann et al. 2000) and the loss of body fat is associated with the decrease in all-cause mortality (Allison et al. 1999b).

2. GENETIC EPIDEMIOLOGY OF OBESITY

One aim of genetic epidemiology of obesity is to evaluate the relative contribution of genetic and environmental factors, and their interaction, to the interindividual variability of body weight. In addition, genetic epidemiology assesses whether the genetic and environmental effects vary with age, gender and other factors. Such studies to determine the genetic basis of obesity probably started in the 1920's and during the following decades, several articles were published on the issue, based on the weight for height data of study subjects (Bouchard 1995). However, in the last 20 years, a large number of studies examining the heritability (h^2) of this complex trait, have used other –and more appropriate- indicators of obesity, such as body-mass index. To date, there is no consensus on the degree of heritability, and the exact estimates are quite varying. This presumably informs us about the complex nature of the trait: its heritability can be influenced by population specific environmental factors varying overtime; also the study design and method of statistical analysis can affect the values obtained. However, the majority of studies agree upon that the genetic factors play a significant role in the causes of individual differences in relative body weight and human adiposity, and furthermore, only a small proportion of genetic variance is attributed to the consequences of assortative mating (Maes et al. 1997).

Twin, adoption and family studies provide the basis for genetic epidemiology studies of obesity. These study models have been recently reviewed (Maes et al. 1997) in the context of body weight, and it was concluded that h^2 estimates from twin studies (0.50-0.90) are usually higher than those from family studies (0.20-0.80), while adoption studies provide somewhat lower estimates of h^2 (0.20-0.60) for BMI. However, it should be emphasized that the estimates of genetic effects are dependent on study definitions and designs: since twin pairs are so well matched by their age and living environment, their mutual correlation in every trait is usually higher than between other family

members. Since virtually every trait varies in expression with age, or a trait may be influenced by different genes at different ages, using twin pairs removes this confounding factor from consideration (Martin et al. 1997). For the onset of a complex disease, susceptibility genes increase the risk, but they are not necessarily required for the development of the disease phenotype. They may only lower the threshold for the outbreak of the particular disease, playing one role in the complicated network of interactions with all the other predisposing factors (Figure 1).

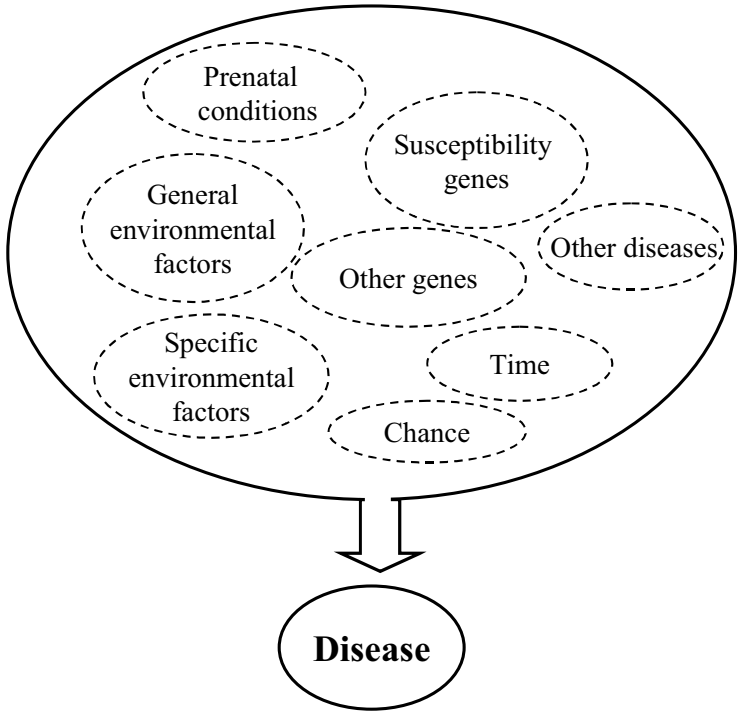

Figure 1. Complex diseases are caused by a combination of genetic and environmental factors, which influence each other differently at different stages of life. When a certain amount of risk factors predisposing to the disease have accumulated, the threshold level is achieved, and the disease develops.

As well as body weight, body height is also a multifactorial characteristic influenced by both genetic and environmental factors (Preece 1996). Body height is the denominator of the BMI, and it is related to BMI non-linearly: the taller the person, the less effect does excess fat have to his/her relative weight. Several environmental factors at different stages of life can contribute to a slower growth velocity. For instance, maternal smoking, malnutrition, diseases, and increased energy expenditure (exercise), can affect the final stature achieved (Kusin et al. 1992; Post and Kemper 1993; Silventoinen et al. 2000). Estimates of the heritability of height have been studied in different populations and they have varied between 0.56 and 0.80 (Crow and Kimura 1970; Roberts et al. 1978; Stunkard et al. 1986a). In studies of Finnish twins and families, the estimates of h^2 have

varied from 0.58 to 0.82, increasing steadily across Finnish birth cohorts born between 1900 and 1957 (Silventoinen et al. 2000; Solomon et al. 1983). This may reflect differences in the standard of living which has improved from decade to decade (Tanner 1992), i.e. the time- and environment specific factors contributing to the gene expression.

2.1 Quantification of familial aggregation and risk

The genetic predisposition, or the relative importance of genetic versus environmental factors as summarized as heritability (h^2), for a disease is observed as an increased risk of the first degree relatives of an affected individual for the disease compared to the risk of more distant relatives, or the risk of general population. Using this viewpoint, a way of measuring the heritability, or the degree of familial clustering of a disease, was proposed by Risch as λ_r (Risch 1990a). Specifically, λ_r is defined as $[P(A \mid R) / P(A)]$, where $P(A)$ is the probability of being affected (i.e. the population prevalence) and $P(A \mid R)$ is the probability of being affected given that one's relative of degree R is affected. Typically, the risk ratio for complex diseases is much lower than for single gene disorders. Also, Risch has shown that the value of λ_r decreases as the relationship between the proband and the relative grows more distant, and the pattern of decrease can be suggestive of the specific model of gene interaction, e.g. additive or multiplicative models (Risch 1990a; Risch 1990b). The risk ratio for siblings is denoted as λ_s, and explicitly, the higher the λ_s, the stronger the genetic effect is concluded to be. Further, if λ_s equals to 1.0, there is no genetic component of the disease. As the λ_r depends on the population prevalence of the trait, its value could vary between different populations although the actual heritability of the disease remains the same.

The λ_r has many useful properties, which make it an important variable in designing and interpreting genetic studies (Bouchard 1995). It can be used, for instance, to determine the number of relative pairs necessary to narrow a chromosomal region containing a putative disease locus to a given length (Kruglyak and Lander 1995). Genetic studies of obesity have triggered studies of heredity and the values of λ_r for obesity to help investigators in study design (Allison et al. 1996). The values of λ_r were calculated for different BMI percentile cutoffs above 50^{th} and by increments of 5, and four different data sets containing different types of relative pairs were used empirically. The results show that λ_s (sibling's relative risk) varies by different data set as well as BMI percentiles. The value of λ_s ranges from 1.18 to 11.70 when BMI percentiles linearly increase from $\geq 50^{th}$ to $\geq 95^{th}$ (Allison et al. 1996). Therefore, it has been concluded that in the genetic studies of obesity it is more efficient to use siblings than other relative pairs and it is more efficient to use the most extreme pairs possible. However, the number of even extreme pairs needed for high-resolution mapping of obesity loci is discouragingly high (Allison et al. 1996; Risch and Zhang 1995).

2.2 Family studies

Family studies including first-, second- or third-degree relatives offer a tool to dissect the familial similarity of a phenotype; is this due to genes, life style, behavioral models or other environmental factors shared by family members. Similarities between family members are usually indicated as correlations. The Quebec Family Study, including 409

large French Canadian families, provided BMI correlations of 0.23 for parent-offspring, 0.26 for siblings and 0.10 for spouse pairs (Bouchard et al. 1988). In addition to relative weight or BMI, several phenotypic variants of obesity, e.g. abdominal fat mass and total fat mass, are shown to have common familial determinants (Rice et al. 1996). However, studies of small families do not easily permit resolution of shared environmental effects from genetic effects. For this reason, adoption and twin studies are used. Complex segregation analysis uses more fully all the information available from large families, but single major genes have not been identified by such analyses (Maes et al. 1997).

2.3 Adoption studies

The aim of adoption studies is to determine the magnitude of environmental and genetic effect on a disease phenotype. In principle, the adoptees are compared to both their adoptive and biological parents, and similarities between the adoptees and their biological parents suggest a genetic component, whereas similarities with their adoptive parents are due to the shared environment. Adoption studies might be biased due to the selection: the adoptive parents and family environment are often chosen to resemble those of the biological parents. Further, adoptees and foster parents may not necessarily be representative of the general population due to the circumstances behind adoption, and often the rigorous screening of potential adoptive parents.

One of the largest complete adoption data sets used to determine the heritability of weight includes 540 adoptees and their biological as well as adoptive parents from Denmark. All the study subjects were divided into four weight classes (thin, normal, overweight and obese) and comparisons produced a significant relation of the weight class of the adoptee and that of his/her biological parent ($p<0.0001$ for mothers and $p<0.02$ for fathers), but no relations was found to the weight class of the adoptive parents (Stunkard et al. 1986b). Another study including 357 adoptees from Iowa, resulted in varying correlation coefficients of 0.08-0.40 for the adoptees and their biological mothers, but still, the correlation between adoptees and their adoptive parents was clearly lower (0.09-0.09).

2.4 Twin studies

The basis for twin studies is the comparison of the similarity of monozygotic (MZ) and dizygotic (DZ) twin pairs. A genetic effect on a disease is suggested, if a higher concordance is found in MZ than DZ twins. As far as complex diseases are concerned, the concordance rate of MZ twins is far less than 100%, typically 20-30%, emphasizing the importance of non-genetic factors in the development of the disease. Deriving the heritability estimations by comparing the similarity of MZ and DZ twins is called the classic twin method. A specific model of studying twins reared apart can combine the best aspects of twin and adoption studies. However, the study material for this design is relatively rare and could be biased by selection.

Several twin studies have shown that genetic factors have an important role in determining body weight or body fatness in adults as well as in children and adolescents (Maes et al. 1997). In addition, the shared family environment does not seem to have a large effect. For relative weight, the MZ twin correlations are approximately twice as high as the DZ correlations ($r_{MZ} \approx 2r_{DZ}$), which indicate an additive genetic component,

i.e. the genetic effect is comprised of additive effects of several genes (Martin et al. 1997).

In a study of twin pairs reared apart, including twin pairs from Finland and other populations, the heritability estimates of BMI varied between 0.50-0.80 (Allison et al. 1996b). According to another study, the heritability of BMI was estimated in twins from Sweden who were reared apart, with an intrapair correlation coefficient of MZ twins of 0.70 for men and 0.66 for women (Stunkard et al. 1990). A large study including 1638 MZ and 2382 DZ American adult twin pairs concluded that the heritability of that portion of weight unrelated to height is substantial, i.e. 0.61 for men and 0.73 for women (Allison et al. 1994). A study using a twin database including US World War II veterans (1974 MZ and 2097 DZ male twin pairs) established the heritability of relative weight in that population to be 0.77-0.84 (Stunkard et al. 1986a). The study using a twin register from Denmark including 535 male and 698 female pairs showed that females had greater heritability for BMI than males, and that heritability in males increases with age (Herskind et al. 1996). The specific heritability estimates for BMI were 0.46 for middle-aged and 0.61 for elderly males, and 0.77 for middle-aged and 0.75 for elderly females. The extent of the genetic effect on body weight on different ages has been studied in Finnish twins and based on the cross-sectional data, the heritability seems to be higher at younger ages whereas the effect of environment appears to increase with age (Korkeila et al. 1991; Pietiläinen et al. 1999). Based on a nationally representative sample of 4884 Finnish adolescent twins including opposite-sex pairs, it was deduced that in adolescence, genetic effects seem to account for over 80% of the interindividual variation of BMI in individuals aged 16 to 17 (Pietiläinen et al. 1999).

3. STRATEGIES FOR SEARCHING FOR GENES OF COMPLEX TRAITS

3.1 The underlying complexities and confounding factors

A complex trait is any phenotype that does not obey the laws of classic Mendelian inheritance, i.e. dominant, recessive or X-chromosomal, attributable to a single gene locus. Several factors contribute to the intricacy of these traits making it more difficult to detect the underlying genes (Lander and Schork 1994). Firstly, mutations in different loci may produce identical phenotypes, for instance when the genes are involved in the same biochemical pathway. This phenomenon is called genetic, or non-allelic, heterogeneity. When different mutations in the same, single gene cause the disease phenotype, it is called allelic heterogeneity, but it does not usually impede gene mapping by linkage analysis. An individual can inherit a predisposing allele, but may not manifest the disease, thus, the penetrance of the disease is incomplete. Alternatively, a phenocopy occurs when an individual gets the disease solely due to the environment without having a predisposing allele. Further, some traits require the existence of several mutations in multiple genes simultaneously before the phenotype develops. The interaction or synergism of genes, a phenomenon called epistasis, plays a significant role in multifactorial traits (Frankel and Schork 1996). Epistasis is said to occur when the combined effect of two or more genes on a phenotype could not have been predicted as the sum of their individual effects. Often, the particular phenotypic outcome could be measured by a quantitative, continuous variable. These traits may represent a so-called

threshold effect, manifested whenever an underlying quantitative variable, influenced by several genes, exceeds a critical threshold, or an effect requiring the joint action of each mutation (Lander and Schork 1994).

The mode of inheritance is far from being fully understood in complex traits. In addition to Mendelian inheritance, there are several other transmission mechanism known, including mitochondrial inheritance, overdominance, genetic imprinting, and anticipation. These models can lead to highly variable transmission rates and require specialized analytical methods. Finally, the high frequency of trait-causing alleles can be confusing; there might be multiple independent copies of the disease allele segregating in the pedigree, and some individuals may be homozygous for that allele, in which case the detection of linkage would be improbable.

3.2 Study designs

3.2.1 Selection of study subjects and population

The task of mapping complex traits can be relieved by careful selection of study subjects, aiming to collect those individuals that most probably are homozygous and possess a strong genetic determinant to their phenotype (Lander and Schork 1994). As was shown by Risch (1990), the higher the relative risk λ_r, the stronger the genetic background of the disease, and thus, the easier it is to find the gene. However, the value of λ_r could be influenced by environmental factors and vary according the population prevalence of the disease, and this should be taken into account when comparing different studies.

An essential aspect is to restrict the phenotype to be as similar as possible between cases. Thereby, the dissection of the clinical picture should be done carefully when collecting the study material. It is advisable to concentrate on individuals with early-onset of disease (Lander and Schork 1994), because the effect of the environment on the phenotype has likely been less extensive and thus, the heritability probably stronger. A family history loaded with several affected relatives may signify a higher λ_r, thus collecting such families could be productive. Further, one should focus on the extreme ends of continuous trait by collecting the cases representing the tails of the distribution, i.e. the most severe and rarest forms of the trait. However, it has been shown that the extreme sampling is not always the most optimal approach for detecting QTLs (Allison et al. 1998a).

It is imperative to control for environmental variation and non-genetic risk factors in the study design of complex diseases (Weiss and Terwilliger 2000) because it is clear that environmental changes can produce dramatic phenotypic changes. In addition to controlling post-natal conditions, pre-natal development can also affect phenotypic expression later in life. Geneticists often disregard the fact that these environmental conditions can be correlated among siblings, leading to inflated estimates of the genetic component of the trait (Weiss and Terwilliger 2000). In studies of MZ twins, this type of confounding factor can be eliminated.

The Finnish population demonstrates the effects of the founder effect and a rapid expansion a few thousand years ago (Nevanlinna 1972; Norio 2000). Compared to a

large, cosmopolitan population, Finns have advantages for use in mapping of genetic diseases, because the number of risk alleles segregating in the population is reduced, and often there is one major mutation descending from a single founder chromosome (Peltonen et al. 1999). Due to this, LD (or nonrandom association of alleles at linked loci) is seen in disease alleles. Using this feature, several genes belonging to the Finnish disease heritage have been successfully mapped (de la Chapelle and Wright 1998; Peltonen et al. 1999). The small, homogenous population could be more suitable also in complex disease mapping providing a higher probability to observe less allelic and locus heterogeneity. In addition, the environmental, cultural and life-style factors tend to be similar in small, isolated populations than in large, mixed populations. One of the most largest projects aiming to collect a nationwide DNA database from an isolated population has been launched in Iceland, although the genetic homogeneity of this population has been questioned (Abbott 2000; Arnason et al. 2000).

However, the usefulness of isolated populations in linkage disequilibrium mapping of complex diseases has been questioned (Kruglyak 1999). Two recent studies have demonstrated that the level of LD for genetic markers does not differ significantly between heterogeneous and genetically isolated populations (Eaves et al. 2000; Taillon-Miller et al. 2000). Specifically, it has been suggested, that even in the Finnish population the number of founder haplotypes may be relatively high, and common disease alleles might be more efficiently identified by drift mapping, i.e. LD mapping, in small old and constant size populations, where the disequilibrium is the result of genetic drift, and is not due to a founder effect (Terwilliger et al. 1998). One population of this kind is Saami, or Lapps, that live in close geographic proximity to the Finns. The LD created by genetic drift does not disappear with time, as opposed to LD in rapidly expanded populations caused by a founder effect.

3.2.2 Candidate gene approach

A candidate gene approach can be applied when some *a priori* information of the disease pathogenesis is known. For example animal models or microarray expression studies can provide several clues to adequate candidate genes. Based on relevant biological knowledge of the pathophysiology, metabolic pathways and/or their disruptions, anatomical sites of signaling routes, tissue expression and different transmitter molecules that are evidently involved in the development of the disease, the most probable candidate genes can be selected. Variants of the selected gene are then tested for association with the disease in populations (case-control study) or for linkage with the disease in families or sibling pairs. Both the single gene and multifactorial disorders can be analyzed by this method, and there are several examples of the successful gene identification obtained by this study approach even in complex diseases. Practically, the candidate gene design means that several polymorphic marker loci either within or flanking the selected gene are chosen for analyses. As far as complex diseases are concerned, failures using this study approach are partly due to the huge variation of polymorphic loci and predisposing genes in a population. Finally, we still do not know all metabolic pathways in humans, so any selection of candidate genes will undoubtedly be insufficient. However, one way to identify potential new candidate genes affecting the disease pathogenesis is to study the differences of gene expression patterns in normal and affected tissues (Gill and Sanseau 2000).

3.2.3 Rodent loci and syntenically conserved human chromosomal regions

Animal studies are an extremely powerful tool in genetic analysis of complex traits. Experimental crosses of mice and rats offer the opportunity to study hundreds of meiosis from a single set of parents, thus removing the problem of genetic heterogeneity and providing possibilities to study far more complex genetic interactions than in human subjects (Lander and Schork 1994). The most significant advantage of animal models is seen in their ability to dissect quantitative traits into discrete genetic factors. Nongenetic noise can be decreased through the use of progeny tests, recombinant inbred strains and recombinant congenic strains (Groot et al. 1992; Lander and Botstein 1989) which allows detection of interacting and modifier genes in single-gene traits as well as in QTL traits.

Studies of animal models may not reveal exactly the same mutations that one would find in humans because the mutation frequency varies between different species, but they will help to discover important genes in the same biochemical pathway or physiological system (Lander and Schork 1994). Animal models have played a very important role in identification of genes implicated in obesity. To date, there are about a hundred obesity predisposing genes and loci identified in mice and rats, and many of these regions have homologues in human. These human counterparts have been under extensive genetic studies in recent years and significant results have been obtained since the cloning of the first obesity genes, agouti and leptin (Bultman et al. 1992; Zhang et al. 1994).

3.2.4 Genome-wide scan

When there is no *a priori* information of the disease pathogenesis -in complex diseases as is often the case, or when a study focuses on finding novel loci contributing to a disease, a genome-wide scan may be useful. The principle of genome screening is to genotype the entire genome with a dense collection of highly polymorphic markers that are evenly spaced, and then calculate an appropriate linkage statistic at each position along the genome, and finally identify the regions in which the statistic shows a significant deviation from what would be expected under independent assortment. Usually a second stage of fine-mapping is used in the regions showing the strongest evidence of linkage in order to narrow down the linked chromosomal region (Holmans and Craddock 1997). To date, microsatellite markers have been used in mapping projects, but single-nucleotide polymorphism (SNP) based microarray technology may offer another option, although one would need a tremendously greater density of SNP than microsatellite markers for adequate screening of the genome (Weiss and Terwilliger 2000).

In genetic studies of complex traits, the study subjects are often well characterized by detailed phenotyping. However, in some cases, only a minority of the collected phenotypic data is utilized in analyses of a particular trait, and much of the information is practically ignored. Thus, there is a waste from both the economical and ecological points of view. A novel strategy of combining genotypic data from several different genome scans to study a yet unanalyzed trait, can be recommended in order not to waste any of the enormous resources already put into genetic mapping projects.

3.3 Statistical methods in genetic studies of complex traits

3.3.1 Association analysis

Association analysis refers to a case-control study based on the comparison of unrelated affected and unaffected individuals from a population (Lander and Schork 1994). It does not require any assumption of the mode of inheritance. The core idea of the study is population correlation rather than cosegregation within a family. A particular allele is said to be associated with the trait if its frequency among the affected is significantly higher than among the unaffected individuals. An important aspect of this type of study is the selection of the control population because careless choice can lead to erroneous results. Controls should always be matched to cases by their ethnic background, age, sex and if possible, environmental influences. To avoid differences in ancestral background, one should always perform association analysis within a homogenous population, and use an "internal control", i.e. parental genotypes, for allele frequencies: those alleles that are not transmitted to the affected individual serve as control alleles in the analysis (Lander and Schork 1994). This method is called haplotype relative risk and can be applied to either alleles or genotypes (Knapp et al. 1993; Terwilliger and Ott 1992). Also the transmission disequilibrium test (TDT) could be carried out to see whether the associated risk allele is transmitted from parent to affected child more often than the "healthy" allele (Spielman et al. 1994). If TDT is used, naturally the differences in ancestral background do not matter. A positive association does not necessarily mean that the associated allele *causes* the disease, rather, it can only occur more often on the chromosomes carrying the actual mutation (be in linkage disequilibrium with it). A good example of a locus with multiple disease-associations without causative relationship is the HLA locus.

3.3.2 Linkage analysis

The basic idea behind linkage analysis is to trace and measure the co-segregation of disease in a family with marker loci by taking information from putative recombination events during meiosis. The traditional parametric linkage analysis has been a successful model for investigating simple Mendelian traits because the power of the method is tied to the accurate specification of the inheritance patterns (autosomal dominant, autosomal recessive). In the standard analysis, model parameters such as allele frequencies and penetrances have to be specified. The indication of linkage is based on the ratio of likelihoods, $L(\theta)/L(½)$, measuring the evidence provided by the pedigree data that the recombination fraction is equal to θ ($<½$, linkage) as opposed to being equal to ½ (no linkage) (Ott 1995). The lod score is the logarithm (base 10) of that ratio of likelihoods, and traditionally a lod score of 3 or higher has been considered as strong evidence for linkage. In the parametric approach, the recombination fraction θ between the disease and marker loci is estimated on the basis of the family data and the mode of inheritance assumed for the trait. If the inheritance model is defined inaccurately, it usually leads to overestimation of the recombination fraction, which is then viewed as a nuisance parameter rather than a good indicator of disease locus position (Ott 1996). If the parametric linkage analysis is used in complex diseases, a loss of information and power can be expected as long as the inheritance model, penetrance, and gene frequency parameters cannot be specifically determined. However, linkage analysis in extended

families has yielded noteworthy results also in complex diseases (Kuokkanen et al. 1996; Pajukanta et al. 1998; Pericak-Vance et al. 1991).

Non-parametric models of linkage analysis (so called model-free analysis) have been developed in order to circumvent the pitfalls caused by wrong assumptions of inheritance patterns. In the non-parametric model, i.e. allele-sharing method, no assumptions are made, rather, it aims to show that random Mendelian segregation of alleles is rejected and thus, affected relatives (e.g. sib pairs) inherit identical copies of chromosomal regions more often than expected by chance (Faraway 1993; Suarez et al. 1978). Specifically, siblings can inherit 0, 1 or 2 alleles that are copies of the same parental allele (i.e. identical-by-descent, IBD) and under random segregation, the expected proportions of these alleles IBD are 25% - 50% - 25% regardless how siblings are sampled. A significant skewing in the distribution towards excess sharing of alleles indicates the presence of linkage when concordant pairs are concerned. If the origin of an inherited allele cannot be unambiguously resolved, e.g. when the parent is homozygous or both parents have the same genotype, or there is simply no parental data available, the inherited allele is called identical-by-state (IBS). As the distinction between these two status (IBD and IBS) is often impossible, the allele-sharing methods require remarkably large number of affected relative/sib pairs to yield conclusive data when compared to traditional linkage analysis with families. If the selected method is based only on the alleles IBS, it is essential to incorporate allele frequency information into the analysis, since more common alleles will be shared by affected individuals simply because they are more common and not because they are coupled or associated with disease alleles.

To increase the power to detect linkage and to position a disease susceptibility gene, multipoint linkage analysis can be used. Using more than a single genetic marker in the analysis is advantageous especially when markers in the particular region are not fully informative. Multipoint analysis can be applied to both parametric (model-dependent) (Lathrop et al. 1984) and non-parametric (model-independent) methods (Kruglyak and Lander 1995; Weeks and Lange 1992).

3.3.3 Quantitative trait locus (QTL) analysis

Quantitative trait locus (QTL) analysis is based on the assumption that the phenotypic similarity between two relatives is correlated with the number of alleles shared at a trait-predisposing locus (Haseman and Elston 1972). In QTL analysis, categorical diagnostic states are not required, rather, one needs a continuously measurable observation (e.g. BMI, blood pressure, insulin sensitivity). Statistically, one may perform regression analysis of the squared difference Δ^2 in a trait between two relatives and the number χ of alleles shared IBD at a locus. When a locus contributing to the variation of the quantitative trait lies near the tested marker locus (i.e. there is a linkage), there will be a negative regression of Δ^2 on the number of alleles shared IBD; for sibs sharing two alleles IBD, Δ^2 will be small, while for sibs sharing no alleles IBD, Δ^2 will be large. A QTL analysis can be employed for sib pairs (Haseman and Elston 1972) and other pedigree relations (Amos and Elston 1989; Olson and Wijsman 1993).

The power for detecting linkage in quantitative trait analysis depends on several factors: the distance between mean phenotype values associated with underlying genotypes, the underlying allele frequencies, the density of marker coverage, heterozygosity of the markers used, and the sampling scheme used to collect data (Ghosh and Schork 1996).

To maximize the power of QTL analysis, several extensions have been developed: a multipoint analysis with two flanking markers (Goldgar 1990) and with all the markers along the genome (Kruglyak and Lander 1995). Another way to overcome the power issue and large sample sizes needed to detect loci of modest effects, is to use selective sampling of sib pairs (Cardon and Fulker 1994), specifically extreme concordant and discordant pairs (Risch and Zhang 1995). Focusing on discordant pairs can reduce the genotyping effort by 10- to 40-fold over conventional designs. Further, all the siblings meeting the proband's criteria in the family should be included in the analysis.

To fully use the inheritance information in mapping data variance components (VC) methods have been recently developed (Amos and Elston 1989; Blangero and Almasy 1997; Fulker and Cherny 1996; Schork 1993). The VC approach have several desirable features (Williams et al. 1997) due to its generality and flexible model capability: any type of pedigree structure is accommodated, and virtually any type of effect or interaction can be incorporated, such as marker-specific effects, residual additive genetic effects and random environmental effects. The VC method exploits all of the available inheritance information in a pedigree of any size, and information from all individuals is used in the analysis, irrespective of affection status or trait value (Williams and Blangero 1999). The fundamental difference between linkage analysis methods and VC methods concerns the distinction of the dependent and independent variables method (Risch and Zhang 1995; Schork 1993): in the VC approach to linkage analysis, the IBD status of relative pairs is accepted as given, and the phenotypic distribution within a pedigree is compared with that expected under a null model of no linkage –thus the IBD information is the independent variable that is used to model a dependent phenotype. In the linkage analysis the situation is reversed. VC methods require assumptions about the phenotypic distribution, and usually, multivariate normality is assumed. However, this is the major drawback of the method: when the distributional assumption is violated, the analysis potentially lacks robustness and exhibits an excess of Type 1 errors (Allison et al. 1999c). There are many important reasons why nonnormality, such as leptokurtosis, of the phenotypic data might occur (Allison et al. 1999c)**,** and since this problem has to be dealt with, a correction constant has been proposed (Blangero et al. 2000) to maintain the strength of the likelihood-based inference.

3.3.4 The issue of statistical significance

In the past, when only simple Mendelian traits were studied by classical two-point linkage analysis, the level of significance was widely accepted to correspond to lod score 3, equivalent to a p-value of 10^{-4} (Chotai 1984). A lod score of 3 means that the observed data is 10^3 –fold more likely to arise under the specified hypothesis of linkage than under the null hypothesis of no linkage. This criteria keeps the chance of false positive at no more than 5% *at a specific locus*, but on the genome-wide level where multiple testing imposes, this significance level corresponds to 9% (Lander and Kruglyak 1995). Thus, in order to avoid excess reporting of false positive "significant" linkage results in the era of genomic screens of multifactorial traits, more stringent threshold lod scores have been proposed. Specifically, Lander and Kruglyak (1995) recommended for sib pair studies that the standard lod score for a suggestive linkage be equal to 2.2 ($p=7\times10^{-4}$), for a significant linkage the lod score should be 3.6 ($p=2\times10^{-5}$) and for a highly significant linkage the lod score should be 5.4 ($p=3\times10^{-7}$). However,

scientist are still encouraged to report all nominal p-values of 0.05 encountered in a complete genome scan, without any claims of linkage though, because there is still a chance that small peaks represent weak but true linkages (Lander and Kruglyak 1995). This information could help other investigators in replicating the regions of interest in their own material, and thus perhaps attain the level of significance. The proposed standard lod score values have been reconsidered (Witte et al. 1996) but also found acceptable and thus been further recommended (Lernmark and Ott 1998). Apart from the chosen cut-off point for lod scores, another mean has been proposed to distinguish between true and false positive linkages. Terwilliger and coworkers (Terwilliger et al. 1997) have shown that true peaks are, on average, wider than false peaks of similar height, and that wider peaks are more likely to contain the disease gene. Further, statistics that use both the width and height of a potential peak may be more powerful than a test based on height alone, because it uses more information (Ott 2000).

5. MONOGENIC FORMS OF OBESITY IN RODENTS AND HUMANS

The field of obesity research has benefitted from several animal models providing a new knowledge and understanding of the complexities underlying the mechanisms of energy balance, thermogenesis, body fatness and regulation of feeding and satiety. The cloning of rodent obesity genes (Table 2) has led investigators to search for the same genes and possible mutations in humans. In a few cases, these studies have been successful. In addition to serving as tools for studying the function, effects and regulation, animal models can help to elucidate the anatomical sites of expression of these genes. The involvement of the hypothalamus in adiposity had been known since 1940 (Hetherington and Ranson 1940), but a new era in obesity research began in the 1990s when the first discoveries of the rodent obesity genes agouti and leptin (Lu et al. 1994; Zhang et al. 1994) generated enormous interest in the interaction between peripheral signals and brain targets involved in the regulation of energy balance and feeding.

In this paragraph, only those animal models of obesity that are widely investigated or most crucial for this thesis work, are described. To date, several other murine models of obesity, mainly knock-out mice and rare spontaneous mutations, have been reported.

Table 2. Single gene mutations in rodent models of obesity and the equivalent human gene. Modified from Chagnon et al. 2000.

Mutation	**Rodent**			**Human**			**References**
	Chr	Gene	Inheritance	Chr	Gene	Gene product	
Diabetes (db)	4	Lepr	Recessive	1p31	Lepr	Leptin receptor	Tartaglia 95
Fat (fat)	8	Cpe	Recessive	4q32	CPE	Carboxy-peptidase E	Naggert 95
Obese (ob)	6	Lep	Recessive	7q31.3	Lep	Leptin	Zhang 94
Tubby (tub)	7	Tub	Recessive	11p15.5	TUB	Insulin signaling protein	Kleyn 96, Noben-Trauth 96, Kapeller 99
Agouti yellow (A^y)	2	A^y	Dominant	20q11.2	ASIP	Agouti signaling protein	Wilson 95

5.1 Leptin (*ob*) and leptin receptor (*db*)

The first evidence for a physiological, homeostatic system for body weight regulation was deduced in the 1950s (Kennedy 1953) and was reinforced after the discovery of recessive mutations, *obese (ob)* and *diabetes (db)* (Ingalls et al. 1950), and subsequent parabiosis studies of these strains by Hausberger and Coleman (Coleman 1973; Hausberger 1959). Their deductions that the *ob* locus was necessary for the production of a humoral satiety factor and that the *db* locus encoded a molecule required for response to this factor were confirmed by the cloning of the *ob* and *db* genes (Tartaglia et al. 1995; Zhang et al. 1994). For mice, the mutations in the *ob* gene cause hyperphagia, early-onset morbid obesity, hypothermia, decreased energy expenditure, hyperinsulinemia and infertility due to the hypothalamic hypogonadism. The *ob* gene product was named leptin (from the Greek *leptos*, meaning thin), because when injected in animals, it led to reduction in food intake, body weight and body fat in addition to the correction of all the metabolic derangements (Halaas et al. 1995; Pelleymounter et al. 1995).

Leptin is synthesized mainly in white adipose tissue but also in gastric epithelium and the placenta (Campfield et al. 1995; Pelleymounter et al. 1995). The plasma leptin levels correlate strongly with leptin mRNA and the mass of the adipose tissue (Considine et al. 1996). The expression of leptin is influenced by several factors, and vice versa, leptin has many stimulatory and inhibitory effects on the neuroendocrine axis in addition to body weight regulation and appetite control (Figure 2) (Ahima and Flier 2000). Its role as the mediator of the adaptation to fasting is manifold. For instance, it serves as an important link between nutrition and the immune system by stimulating inflammatory response and T-cell proliferation (Lord et al. 1998), and it is necessary for the maturation and proper function of the reproductive axis (Chehab 1997; Chehab et al. 1996). Leptin stimulates gonadotropin-releasing hormone (GnRH) (Finn et al. 1998) and corticotropin-releasing hormone (CRH), and a fall in leptin levels suppresses thyroid function by

decreasing thyrotropin-releasing hormone (TRH) formation (Legradi et al. 1997). Leptin inhibited glucose-responsive neurons in hypothalamus, and insulin secretion from pancreatic β-cells is decreased through leptin's effects on ATP-sensitive potassium channels (Harvey et al. 1997; Spanswick et al. 1997).

Soon after the discovery of leptin, the leptin receptor gene (Ob-R) was isolated in *db/db* mice (Tartaglia et al. 1995) that have a similar phenotype to *ob/ob* mice in addition to leptin insensitivity. Ob-R mRNA has multiple splice variants that encode at least six leptin receptor isoforms (Lee et al. 1996), and only the long isoform (Ob-Rb) contains the intracellular motifs required for signal transduction and transcription activation pathway (Ghilardi et al. 1996). The short isoforms are expressed in various tissues, e.g. the choriod plexus, vascular endothelium, kidney, liver and gonads (Ahima and Flier 2000). The long form of the leptin receptor polypeptide is expressed in hypothalamic regions implicated in feeding behavior and energy balance, and it is colocalized with the neuropeptide mediators of leptin action (Figure 2), such as neuropeptide Y (NPY) and proopiomelanocortin (POMC), agouti-related peptide (AgRP) and cocaine- and amphetamine-regulated transcript (CART) (Baskin et al. 1999; Elmquist et al. 1999).

Leptin-sensitive neurons may control feeding by influencing the expression of orexigenic peptides, for instance melanin-concentrating hormone (MCH) and hypocretin. Further, anorexigenic peptides such as POMC and CART are increased in response to leptin administration (Kristensen et al. 1998). Potential transmitters of leptin action in the brain also include corticotropin-releasing hormone (CRH), cholecystokinin (CCK), .glucagon-like peptide-1 (GLP-1), urocortin, bombesin and serotonin (Flier and Maratos-Flier 1998).

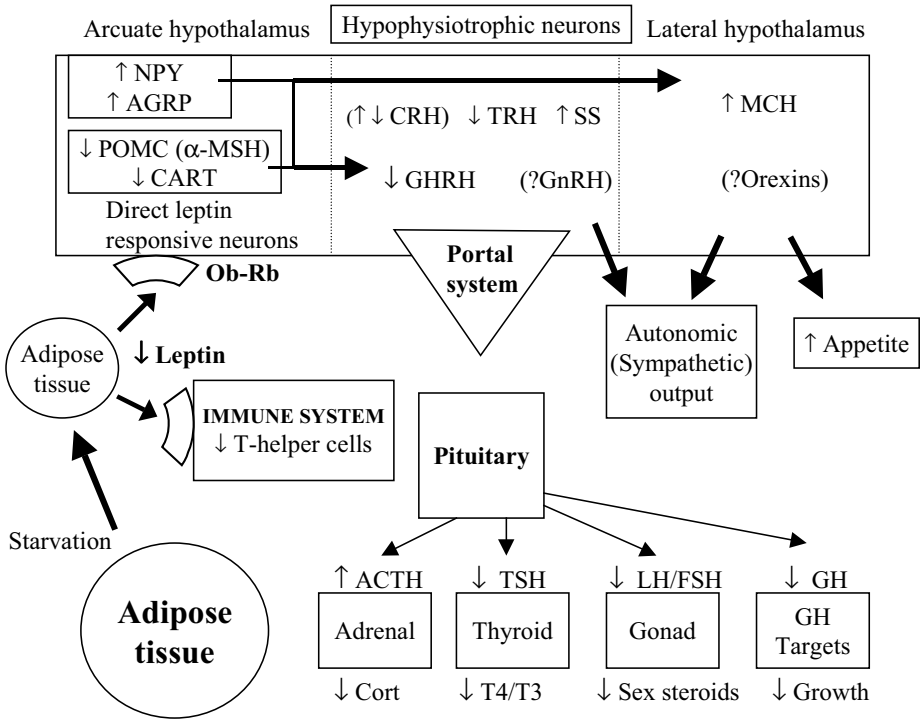

Figure 2. Role of leptin in the adaptation to starvation. The fall in levels of leptin with starvation results in an increase in neuropeptide Y (NPY) and agouti-related peptide (AGRP) levels, and a decrease in pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) levels in the arcuate hypothalamic nucleus. NPY and AGRP stimulate feeding, whereas α-melanocyte stimulating hormone (α-MSH), a product of POMC, and CART inhibit feeding. These neurons also project to the lateral hypothalamus and regulate the expression of the melanin-concentrating hormone (MCH), a major stimulator of feeding. Additionally, leptin targets in the arcuate hypothalamus respond to low leptin levels by regulating the neuroendocrine axis and decreasing sympathetic nervous output. A fall in leptin also leads to suppression of immune function. CRH (corticotropin-releasing hormone), TRH (thyrotropin-releasing hormone), GHRH (growth hormone-releasing hormone), SS (somatostatin), GnRH (gonadotropin-releasing hormone), GH (growth hormone). Modified from Ahima and Flier (2000).

Since the leptin gene and its receptor were identified in rodents, their human counterparts have been targets for intensive genetic study. Equivocal reports exist of evidence of linkage and no linkage between obesity and chromosomal region 7q31.3 containing the human OB (i.e. LEP) gene (Clement et al. 1996; Norman et al. 1996; Reed et al. 1996) as well as the OB-R region in chromosome 1p31 (Chagnon et al. 1997; Gotoda et al. 1997; Thompson et al. 1997). The quantitative phenotype, serum leptin level, has been significantly linked to chromosome 2p21 in a genome scan of Mexican-Americans (Comuzzie et al. 1997), to chromosome 5q-cen in a French population (Hager et al. 1998) and to chromosome 6p12-21 in Pima Indians (Walder et al. 2000). The mutations in leptin and leptin receptor genes causing recessive obesity syndrome are extremely rare in human subjects. To date, mutations in the leptin gene has been reported in only two pedigrees: one consanguineous family of Pakistani origin (Montague et al. 1997) and the other family of Turkish origin (Strobel et al. 1998). The

individual mutations of the LEP gene are given in Table 4. These leptin-deficient human subjects demonstrate early-onset morbid obesity, hyperphagia and hypothalamic hypogonadism, but unlike the *ob/ob* mice, they are not hypothermic, hyperglycemic or hyperinsulinemic. Only one consanguineous family of Kalibian origin has been described with mutations in the leptin receptor gene (Clement et al. 1998) so far (Table 3). In addition to early-onset morbid obesity, hyperphagia and hypothalamic hypogonadism, the phenotype of these subjects includes impaired secretion of thyrotropin and growth hormone. Unlike the *db/db* mice, the human leptin receptor deficiency is not associated with hyperglycemia, hypothermia or hypercorticism.

5.2 Agouti (A^{vy}), agouti-related protein (AgRP), agouti signaling protein (ASP) and mahogany (*mg*)

Although the genetic defects of *agouti* and related mouse models are involved in the over-expression of melanocortin antagonists, and thus could be included in the next paragraph of melanocortinergic signaling, they are described separately here.

Agouti (*A/a*) mouse is the oldest known mouse model of obesity. The autosomal dominant *lethal yellow* (A^{y}) mouse was identified already at the turn of the last century and *viable yellow* (A^{vy}) was initially described in the 1950s (Silvers and Russel 1979). Animals heterozygous for the *agouti* locus were first investigated for their golden yellow coat color, but the phenotype was further characterized by a syndrome of late-onset obesity associated with hyperphagia, hyperinsulinemia, hyperglycemia and increased linear growth as well as an increased propensity for developing tumors (Bultman et al. 1992; Michaud et al. 1994). Cloning and analyses of the *agouti* gene revealed that the phenotype is caused by inherited promotor rearrangements at the agouti locus that result in constitutive ectopic expression of the *agouti* peptide (Bultman et al. 1992; Duhl et al. 1994; Miller et al. 1993).

Normally, *agouti* is a paracrine-signaling factor, that is secreted only adjacent to melanocytes within hair follicle where it blocks the binding of melanocyte-stimulating hormone (α-MSH) to melanocortin receptor 1 (MC1-R) and thus regulated hair pigmentation (Lu et al. 1994). In addition to being a potent antagonist of MC1-R, *agouti* strongly antagonizes the action of melanocortin peptides at the MC4-R (Figure 3), and to a lesser degree at the MC2-R, but not at the MC3-R or the MC5-R. (Yang et al. 1997). The human homolog of mouse *agouti* gene, agouti signaling protein (ASP, ASIP) was positionally cloned from the human chromosome 20q11.2 (Kwon et al. 1994), and was shown to be expressed in adipose tissue, heart, ovary, testis and foreskin. ASP has similar pharmacological profile as the murine *agouti*: it is a potent antagonist at human MC1- and MC4-receptors, relatively weak at MC3- and MC5-R. However, competitive antagonism is apparent only toward MC1-R (Yang et al. 1997).

The obesity phenotype was hypothesized to result when over-expressed *agouti* protein chronically antagonizes melanocortin receptor 4 in hypothalamic areas known to be involved in the control of food intake and body weight. The most compelling evidence to support this hypothesis were the findings that targeted disruption of MC4-R signaling results in knockout mice with a phenotype similar to A^{y} syndrome (Huszar et al. 1997) and central administration of MC4-R agonists and antagonists stimulate and inhibit

feeding behavior, respectively (Fan et al. 1997). Further proof came from isolation of the agouti-related protein (AgRP) from rodents and humans (Ollmann et al. 1997; Shutter et al. 1997), a peptide sharing high sequence homology with *agouti*, and showing strong binding affinity to central MC3-R and MC4-receptors but very little or no activity at MC5- and MC1-receptors. Subsequently, it was shown that ubiquitous overexpression of AgRP results in an obese mouse with a phenotype identical to the *agouti* mouse, apart from the normal coat color (Graham et al. 1997; Ollmann et al. 1997).

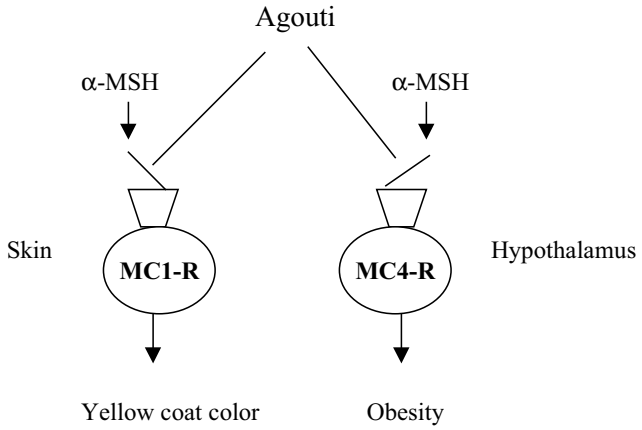

Figure 3. Agouti protein antagonism of MC1-R and MC4-R blocks the effect of α–melanocyte stimulating hormone (α–MSH) and leads to yellow coat pigmentation and obesity. Modified from Fisher et al. (1999).

Unlike *agouti*, the expression pattern of mouse and human AgRP is strikingly alike: primarily in the hypothalamus and adrenal medulla, and at lower levels in the testis, lung and kidney. In view of the similar distribution of its "high affinity" receptors MC3- and MC4-R, it seems likely that AgRP controls their function in the brain in a similar way as *agouti* modulates MC1-R signaling in skin.

Homeostatic regulation of the central melanocortin system may be achieved through changes in the bioavailability of the antagonist (AgRP) rather than the agonist (α-MSH) (Dinulescu and Cone 2000). The key role of AgRP in regulation of energy homeostasis is further supported by demonstrations that it is able to stimulate feeding when administered centrally, and prevents the reduction in food intake caused by administration of α-MSH (Rossi et al. 1998). In addition, AgRP mRNA in the hypothalamus is inhibited by leptin and stimulated by fasting (Mizuno and Mobbs 1999). Two autosomal mutations mahogany (*mg*) and mahoganoid (*md*), natural suppressors of *agouti* action, were only recently shown to suppress not only the pheomelanin synthesis in the skin, but the whole obesity syndrome of A^y mice (Dinulescu et al. 1998). Mahogany is thus involved in the interaction of *agouti* and melanocortin receptors 1 and 4. Since *mg* protein has physiological effects also in the absence of A^y mutation, it is argued that it facilitates the binding of AgRP to MC4-R as well. A chronic increase in MC4-R signaling could explain the hypermetabolic behavior of *mg* animals. Based on the genetic studies, *mg* cannot suppress the phenotype of either MC1-R or MC4-R

knockout, thus it would be positioned upstream or at the same level of these receptors (Nagle et al. 1999).

To date, no evidence of human obesity related to mutations in ASP or AgRP genes has been reported. Genetic studies have failed to show any linkage to them as well (Hasstedt et al. 1997; Norman et al. 1996).

5.3 Genes of the melanocortinergic signaling pathway

In the last five years, the important role of melanocortin signaling in the control of appetite and body weight has been elucidated with murine and human genetic models. The impact of melanocortins as catabolic mediators of leptin action and satiety-inducing agents has become evident. In the following paragraph, the melanocortin pathway and its disruptions are described with examples of genetic defects at the level of synthesis, processing and receptor signaling.

5.3.1 Pro-opiomelanocortin (POMC) and melanocyte-stimulating hormone (α-MSH)

The first suggestions that melanocyte-stimulating hormone (α-MSH) and adrenocorticotrophic hormone (ACTH) might play a significant role in central nervous system functioning come from the 1950s (Ferrari 1958), but the observations of their inhibition of feeding were made much later (Panskepp et al. 1976; Poggioli et al. 1986). α-MSH and ACTH are derived from a precursor peptide, pro-opiomelanocortin (POMC), which is expressed in skin, the pituitary, hypothalamic neurons and the immune system (Bertagna 1994). In these tissues, POMC is cleaved into smaller peptides including adrenocorticotrophic hormone (ACTH), β-endorphin and α-, β- and γ-melanocyte-stimulating hormones (MSH), by specific endoproteases (convertases). Anterior pituitary corticotrophs express prohormone convertase 1 (PC1) and cleave POMC to ACTH, while melanotrophs express PC2 and cleave ACTH further to yield α-MSH. The physiological role of the cleaved peptides is only partly known in mice and humans. ACTH binds to MC2-R in the adrenal cortex and is an irreplaceable factor in adrenal steroidogenesis (Axelrod and Reisine 1984) and β-endorphin interacts with opioid receptors. MSH binds to melanocortin receptors centrally and peripherally: in addition to its effect on feeding inhibition by MC3- and MC4-R, it has also been implicated to skin pigmentation in humans, since variants of human MC1-R are associated with red hair and fair skin (Valverde et al. 1995).

The POMC-expressing neurons in the arcuate nucleus project extensively to the brain areas thought to regulate feeding and at least 40% of these neurons express mRNA for leptin receptor (Cheung et al. 1997). POMC expression is increased by leptin and diminished in hypoleptinaemic states such as fasting or in *ob/ob* mouse (Thornton et al. 1997). A newly discovered anorexic peptide CART (cocaine- and amphetamine-regulated transcript) is also expressed in POMC-containing neurons (Kristensen et al. 1998) and appears to co-operate with POMC in mediating the appetite suppression effects of leptin.

The evidence of POMC involvement in human obesity came from two unrelated children with syndrome of hypocortisolism (no detectable plasma cortisol or ATCH), hyperphagia and progressive obesity associated with red hair and fair skin (Krude et al.

1998). One proband was compound heterozygous for two mutations preventing ACTH and α-MSH synthesis, the other was homozygous for a nucleotide transversion in the 5'-UTR (Table 3). The normal phenotype of the heterozygous parents indicated a recessive mode of inheritance. A putative role for POMC in more common forms of human obesity has been supported by positive reports of linkage studies. Genome-wide QTL analysis of Mexican-Americans found a significant linkage to a locus on chromosome 2p21, which encompasses the POMC gene (Comuzzie et al. 1997), and estimated that this locus accounted for 47% of the variation of the serum leptin levels. Studies on French and African-Americans subjects have reported the same association (Hager et al. 1998; Rotimi et al. 1999).

The mouse model of POMC deficiency has a similar phenotype as human subjects: obesity, defective adrenal development and altered pigmentation (Yaswen et al. 1999). Detailed examination revealed that the weight gain was accompanied by both an increase in body length and a large increase in serum leptin levels. Further, when mutant mice were raised on a high-fat diet they gained weight faster than mice raised on standard chow, and on either diet, the mutants had a greater food intake. This indicates, that POMC-derived peptides mediate both food intake and bodily food deposition, i.e. they lack the ability to properly adjust their metabolism to decrease food intake with a higher caloric supply. When POMC mice were treated with α-MSH agonist, they lost 46% of their excess weight, and gained it back when the injections were stopped.

Table 3. Cases of human obesity presumably caused by single gene mutations in leptin receptor (LEPR), pro-opiomelanocortin (POMC), prohormone convertase 1 (PC1), leptin (LEP) and melanocortin receptor 4 (MC4R). Modified from Chagnon et al. (2000).

Gene	Chr	Mutation	Sex	Age	BMI (kg/m^2)	Reference
LEPR	1p31	G→A in exon 16	F	19	65.5	(Clement et al. 1998)
			F	13	71.5	(Clement et al. 1998)
			F	19	52.5	(Clement et al. 1998)
POMC	2p23	G7013T & C 7133 in exon 3	F	3	NA	(Krude et al. 1998)
		C3804A in exon 2	M	7	NA	(Krude et al. 1998)
PC1	5q15-q21	Gly483Arg A→C+4 in intron 5	F	3	NA	(Jackson et al. 1997)
LEP	7q31	G398delta in codon 133	F	8	45.8	(Montague et al. 1997)
			M	2	36.6	(Montague et al. 1997)
		C→T in codon 105 in exon 3	F	6	32.5	(Strobel et al. 1998)
			M	22	55.8	(Strobel et al. 1998)
			F	34	46.9	(Strobel et al. 1998)
			F	30	54.9	(Ozata et al. 1999)
MC4R	18q21.3	CTCT nt 631-634 deletion in codon 211	M	4	28	(Yeo et al. 1998)
			M	30	41	(Yeo et al. 1998)
			F	20	42.1	(Hinney et al. 1999)
			F	43	37.6	(Hinney et al. 1999)
		GATT insertion at nt 732 in codon 246	F	58	51	(Vaisse et al. 1998)
			F	35	57	(Vaisse et al. 1998)
			M	24	33	(Vaisse et al. 1998)
			F	11	30	(Vaisse et al. 1998)
		C105A nonsense mutation in codon 35 (Tyr35X)	F	10	31.1	(Hinney et al. 1999)
			F	17	45.9	(Hinney et al. 1999)
			F	31	48.2	(Hinney et al. 1999)
		GT insertion in codon 279	M	36	NA	(Farooqi et al. 2000)
			F	8	NA	(Farooqi et al. 2000)
		C271Y	F	40	NA	(Farooqi et al. 2000)
			F	8	NA	(Farooqi et al. 2000)
		N62S (homozyg.)	M	17	NA	(Farooqi et al. 2000)
			M	16	NA	(Farooqi et al. 2000)
			F	14	NA	(Farooqi et al. 2000)
		Thr11Ser	F	47	45	(Vaisse et al. 2000)
		Ile170Val	F	28	50	(Vaisse et al. 2000)
		Arg165Trp	F	49	64	(Vaisse et al. 2000)

NA= not available; F= female; M= male

*5.3.2 Fat (*fat/fat*), carboxypeptidase E (CPE) and prohormone convertase 1 (PC1)*

Autosomal recessive mouse mutation *fat* (*fat/fat*) was found to elicit adult-onset obesity, infertility but not sterility, and hyperinsulinemia with hypertrophy and hyperplasia of the islets of Langerhans (Coleman and Eicher 1990). Later, the phenotype was specified to be hyperproinsulinaemic, which led to the idea, that the defective gene could be involved in the processing of prohormones including proinsulin. The *fat* mutation was subsequently mapped to carboxypeptidase E gene (CPE), an exopeptidase, which co-operates with prohormone convertases in the proteolytic maturation of many hormone and neuropeptide precursors (Naggert et al. 1995). A missense mutation ($Ser^{202}Pro$) identified in CPE results in abolished enzyme activity in not only islet tissue, but also in the pituitary and brain. As transgenic replacement of CPE activity in the islets of fat animals did not correct obesity, it was deduced that excess weight gain results from defects in exopeptidase processing of a number of neuroendocrine or endocrine prohormones and not islet dysfunction (Naggert et al. 1995). To date, the cause of obesity in *fat/fat* mice is still a mystery, although the defective processing of several neuropeptides offers a tantalizing explanation: the CPE dysfunction will affect numerous neuropeptides controlling satiety, e.g. glucagon-like peptide 1 (GLP1), neurotensin, pro-opiomelanocortin (POMC), as well as orexigenic neuropeptide Y (NPY) and melanin concentrating hormone (MCH) (Yeo et al. 2000). The human CPE gene has been assigned to chromosome 4 (Hall et al. 1993), but thus far, no human mutations have been reported.

In 1995, O'Rahilly and co-workers (O'Rahilly et al. 1995) reported a woman with a novel syndrome suggestive of defective prohormone processing. Her phenotype included extreme childhood obesity (36 kg at 3 years), reactive hypoglycemia and hypogonadotrophic hypogonadism as well as greatly increased ratios of proinsulin to insulin and POMC to ACTH. Altogether, her clinical picture was remarkably similar to *fat/fat* mouse. Given the particular form of proinsulin in her plasma, a defect in prohormone convertase 1 (PC1) seemed most likely, and subsequently, this patient was found to be compound heterozygous for deleterious mutations in the PC1 gene (Table 4) (Jackson et al. 1997). Each of the patient's four children carried one mutated PC1 allele, but were clinically unaffected.

The expression of PC1 is restricted to the neuroendocrine tissues where it acts proximally to CPE in the pathway of post-translational processing of prohormones and neuropeptides. PC1 causes preferential cleavage at pairs of basic amino acids from their substrates in acidic vesicles, and subsequently, the exposed C-terminal basic residues of PC1 are removed by carboxypeptidases (Leibel 1997). Other convertases may compensate for reduced PC1 activity, e.g. PC2, PC5, as in the *fat/fat* mouse, where other carboxypeptidases partially compensate for deficient CPE activity (Naggert et al. 1995). Interestingly, mice with null PC2 alleles are not obese although PC2 is shown to be necessary for the formation of α-MSH from POMC as well as for processing of other anorexic peptides such as corticotropin releasing hormone (CRH) and neurotensin (Perone et al. 1996; Zhou et al. 1993). To date, the exact physiologic mechanism responsible for the human obesity in PC1 deficiency is not known, further investigations are eagerly awaited.

5.3.3 Melanocortin receptors 3 and 4 (MC3-R, MC4-R)

There are five known melanocortin receptors based on the sequence homology ranging from 35-60% homology between the members (Cone et al. 1996) and they all belong to the G-protein coupled receptor family. Firstly, the MC1-R was recognized in melanocytes, and MC2-R was identified from the adrenal cortex, but very little was known about the function of MC3- and MC4-R at the time when the *agouti* gene was cloned in 1992. After the puzzling observations that *agouti* protein is a potent antagonist of not only MC1-R but also MC4-R and that MC4-R is largely expressed in the brain, the picture became clear. The absolute role of MC4-R in the development of obesity was finally proved with the $MC4R^{-/-}$ knockout (KO) mouse recapitulating the A^y syndrome (Huszar et al. 1997). The gene disruption in KO-mice resulted in maturity-onset obesity, hyperphagia (50% increased food intake), hyperinsulinaemia, hyperglycaemia, adipocyte hypertrophy and increased linear growth of app. 10%. Heterozygotes for the null MC4R allele exhibit a phenotype intermediate between that of wild-type and homozygous littermates. The extent of hyperglycemia and hyperinsulinaemia in KO-mice is gender dependent, with males exhibiting more severe elevation than females in both cases. Additional evidence was provided by studies showing that, in a dose-dependent manner, MC4-R integrates an agonist (food inhibition) signal provided by α-MSH (Fan et al. 1997) and antagonist (food intake) signal provided by AgRP in neurons distinct from the POMC neurons in the hypothalamic arcuate nucleus (Graham et al. 1997; Ollmann et al. 1997).

Human MC4-R is a single exon gene localized in chromosome 18q22 and encodes a 332-amino acid protein (Gantz et al. 1993). The impact of MC4-R on human obesity has been studied eagerly since 1997 and the first reports gave inconclusive results. Linkage and association with obesity phenotypes (BMI, fat mass, %fat) were detected for both MC4-R and MC5-R in the Canadian Family study (Chagnon et al. 1997), the latter showing more significant linkage. A missense variance $Val^{103}Ile$ was first detected in British males (Gotoda et al. 1997) but was not associated with obesity. An amino acid substitution severely impairing the receptor function ($Ile^{137}Thr$) associated with obesity as well as several allelic variants have been reported in morbidly obese subjects (Gu et al. 1999). The first impressive evidence of dominantly inherited human obesity associated with mutations in the MC4-R gene were reported in 1998 when systematic mutation screening first revealed three different haploinsufficiency mutations in European populations (Table 3) (Hinney et al. 1999; Vaisse et al. 1998; Yeo et al. 1998). The following large-scale sequencing studies have established the MC4-R mutations heterogeneous and the most frequent (3-4%) cause of monogenic human obesity to date (Farooqi et al. 2000; Vaisse et al. 2000).

The clinical features of heterozygous MC4-R mutant subjects include hyperphagia, hyperinsulinemia, no impairment in adrenal or reproductive functions, a tendency toward a tall stature, and a greater degree of adiposity in females. Further, these human carriers seem to be relatively healthy, they display a common, non-syndromic form of obesity, which can be associated also with moderate obesity especially in males (Sina et al. 1999). In MC4-R deficient children, increased growth velocity, greater bone mineral density and advancement of bone age were observed, and the pubertal development was intact (Farooqi et al. 2000). All these characteristics are consistent with the murine data, and suggestive of haploinsufficiency rather than dominant negativity. The variable penetrance and expressivity of obesity in heterozygous individuals argues that MC4-R

acts in concert with a number of other genes to regulate energy storage (Cone 2000).

The first description of homozygous MC4-R mutations ($N^{62}S$) in humans with a recessive mode of inheritance, was a large consanguineous family of Pakistani origin (Farooqi et al. 2000). In this pedigree, none of the heterozygous parents had BMI ≥ 30 kg/m^2. Functional studies of this mutation provided explanation: in contrast to dominantly inherited missense and nonsense mutations, $N^{62}S$ mutation retains some capacity to signal to cAMP generation. Thus, one partially functioning $N^{62}S$ allele appears to be sufficient to prevent the development of morbid obesity. The homeostatic control of body weight seems to be sensitive to quantitative variation of MC4-R expression, i.e. MC4-R may represent a tightly regulated control point in the system. Pharmacologically thought, MC4-R represents an exciting therapeutic target, since it has an anatomically restricted expression pattern and its dysfunction gives a quite "pure" obesity phenotype (Yeo et al. 2000).

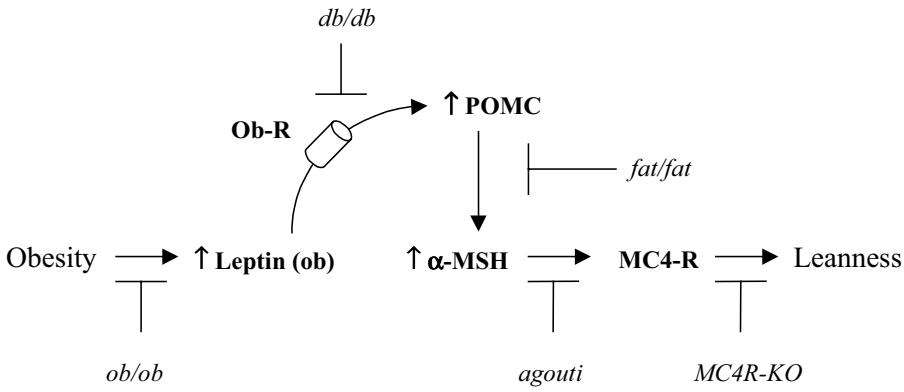

Figure 4. Proposed model for MC4-R in the leptin signaling pathway. The sites of disruption of the pathway that occur in different mouse models are shown. Modified from Fisher et al. (1999).

Melanocortin receptor 3 is primarily found in the CNS, but expressed also in placenta, gut, pancreas, adipose tissue, skeletal muscle, kidney and heart (Wikberg 1999). The human MC3-R maps to chromosome 20q (Magenis et al. 1994) and a QTL analysis has found a linkage between this locus and subcutaneous fat mass as well as fasting insulin levels (Lembertas et al. 1997). By contrast, no linkage of obesity to MC3-R was found by Li et al. (Li et al. 2000) The involvement of MC3-R in the regulation of energy homeostasis has been evaluated recently with the creation of a $MC3R^{-/-}$ mouse and a double KO-mouse $MC3R^{-/-} \times MC4R^{-/-}$ (Chen et al. 2000). Despite being hypophagic and maintaining normal metabolic rate, $MC3R^{-/-}$ mice have increased fat mass, reduced lean mass and higher feed efficiency (i.e. ratio of weight gain to food intake). They are hyperleptinaemic (due to the increased fat mass), they have normal corticosterone and thyroxine levels, and male mice develop mild hyperinsulinaemia. Furthermore, the bone and body length were shorter than in their wild-type littermates, but the bone mineral content was normal. Increased fat mass was predominantly due to adipocyte hypertrophy

as in mice expressing *agouti* or AgRP – natural antagonists of MC3- and MC4-R - ectopically (Graham et al. 1997). When $MC3R^{-/-}$ animals were exposed to regular or high-fat diet, they consumed normal or decreased amounts of food, yet gained more body weight than either wild-type or heterozygous $MC3R^{-/+}$ mice. Thus, the increased feed efficiency, not hyperphagia, contributes to greater fat mass and reduced lean mass, i.e. nutrients are preferentially partitioned into fat at the expense of lean mass (Chen et al. 2000).

To better understand the differences between $MC3R^{-/-}$ and $MC4R^{-/-}$ mice, double KO-mice $MC3R^{-/-} \times MC4R^{-/-}$ was created (Chen et al. 2000). Because these mice were significantly heavier than mice lacking only MC4-R, it was concluded that MC3-R and MC4-R serve non-redundant roles in the regulation of energy homeostasis. One possible explanation for the finding is that double KO-mice eat excessively, due to the MC4-R deficiency, and store ingested calories more efficiently, due to the absence of both receptors (Cummings and Schwartz 2000). Mutations at the MC3-R locus could contribute in part to the "thrifty genotype", a putative collection of alleles that were positively selected over evolution because they promote fat storage in times of plenty, thus protecting against times of famine (Neel 1999). Leptin and its receptors are poor thrifty gene candidates, as their mutation cause also infertility, thus preventing positive selection. In contrast, melanocortin receptors are perfect candidates, as their allelic variations associated with haploinsufficiency should promote fat storage without impairing fertility (Cummings and Schwartz 2000).

5.4 Tubby (*tub*)

Tubby (*tub/tub*) is a spontaneous autosomal recessive mutation in mice that causes slowly progressing maturity-onset obesity, insulin resistance as well as cochlear and retinal degeneration (Coleman and Eicher 1990; Ohlemiller et al. 1995). *Tubby* mice are not sterile, but they become infertile after significant obesity develops. The insulin resistance develops gradually with their weight gain but does not progress to overt diabetes. Despite hyperactivity of their islet β-cells, *tubby* mice have almost normal glucose levels, they are also normocorticoidaemic.

The identity of the mutated *tub* gene was determined by positional cloning (Kleyn et al. 1996; Noben-Trauth et al. 1996) and it was shown to be a member of a novel gene family encoding proteins of unknown function that are highly conserved across species (North et al. 1997). The *tub* mutation was mapped to mouse chromosome 7 and the human homolog of *tub* to the syntenic region of chromosome 11p15 (Chung et al. 1996). The *tub* mutation is a G→T transversion resulting in an aberrant transcript where the C-terminal 44 amino acids are substituted with 24 different amino acids encoded by the intron. The aberrant *tub* mRNA is expressed at elevated levels in *tubby* mice (Kleyn et al. 1996) but no mutant Tub protein could be detected in brain lysates of these mice (Stubdal et al. 2000). This indicates a defect at the posttranscriptional level, generation of an unstable protein and thus, reflects an attempt to upregulate the Tub expression. Normally, the Tub protein is expressed strongly in the key hypothalamic nuclei that have been implicated in the central control of energy homeostasis. It is also strongly expressed in the eye and testis, and moderately expressed in ovary, lung, thymus, intestine and adipose tissue (Kapeller et al. 1999; Noben-Trauth et al. 1996). The expression of Tub protein is not significantly altered in *ob*, *db* or MC4R -deficient mouse models of obesity

(Stubdal et al. 2000).

It was demonstrated that *tubby* is a loss-of-function mutation by generation of a *tub*$^{-/-}$ knockout mouse which replicated the whole spectrum of *tubby* phenotype (Stubdal et al. 2000). Further, it was shown that loss of photoreceptors in the retina in these mice occurs by apoptosis. Strikingly, in humans, a mutation in Tub-like protein 1 (TULP1) belonging to the *tub* gene family, was found to cause an autosomal recessive form of retinitis pigmentosa (RP14) (Banerjee et al. 1998; Hagstrom et al. 1998). It is possible, that late-onset obesity of *tubby* mice could be due to the apoptotic loss of cells in the hypothalamic nuclei, but thus far, no cellular alterations in the hypothalamus of these mice have been found (Stubdal et al. 2000). The first evidence of the biological function of the Tub protein implicates it in intracellular signaling by insulin (Kapeller et al. 1999). It was shown that insulin and insulin-like growth factor 1 (IGF-1) can induce tyrosine phosphorylation of Tub, and upon phosphorylation, Tub associated selectively with the Src homology 2 (SH2) domain –containing proteins. Thus, Tub may function as an intracellular adaptor protein, and in *tubby* mice, the central insulin-mediated regulation of energy homeostasis may be disrupted resulting in weight gain and in insulin resistance (Kapeller et al. 1999). By structure-based functional analysis Tubby-like proteins were suggested to be a unique family of bipartite trancription factors (Boggon et al. 1999).

Interestingly, the *tubby* phenotype resembles human syndromes with sensory deficits and obesity such as Alström's and Bardet-Biedl (BBS) that are characterized by combined hair cell and retinal photoreceptor loss accompanied by infertility and moderate late-onset obesity (Bray 1995; Kwitek-Black et al. 1993). To date, six loci for BBS have been mapped (Table 4) and of these, only one gene for BBS has been conclusively identified thus far (Katsanis et al. 2000). This gene, MKKS (from McKusick-Kaufman syndrome), is located in human chromosome 20p12 and encodes a chaperonin-like protein. It was deduced that a complete loss of function of the MKKS product leads to an inability to fold a range of target proteins and thereby generates the clinical picture of Bardet-Biedl syndrome (Katsanis et al. 2000).

5.5 Serotonin receptor 2C (5HT2CR)

Serotonin (5-HT) is a monoaminergic neurotransmitter presumed to modulate several motor, behavioral and sensory processes through a large family of G-protein coupled receptor subtypes that are widely expressed in the brain and spinal cord (Peroutka et al. 1990). The serotonergic system in the brain has been strongly implicated in the regulation of eating behavior and body weight by studies demonstrating the anorectic effect of drugs that stimulate postsynaptic 5-HT receptors (Blundell et al. 1995; Heisler et al. 1998; Leibowitz and Alexander 1998; Simansky 1996). The human 5-HT_{2C} receptor subtype has been cloned (Saltzman et al. 1991) and localized in chromosome Xq24 (Milatovich et al. 1992). More specifically, this receptor subtype has been shown to play the key role in appetite regulation (Baxter et al. 1995; Curzon et al. 1997): a treatment of obese subjects with 5-HT_{2C} receptor agonist decreased subjective feelings of hunger and caused weight loss (Sargent et al. 1997). 5-HT_{2C} receptors have also been implicated in eating disorders such as binge eating and bulimia nervosa, where dysfunction and reduced response of this receptor has been reported (Dourish 1995; Jimerson et al. 1997; Wolfe et al. 1997).

To investigate the function of 5-HT_{2C} receptor, knock-out (KO) mice 5HT2CR$^{-/-}$ were created (Nonogaki et al. 1998; Tecott et al. 1995). These mutant mice are hyperphagic and develop "middle-aged" –onset obesity and as these mice age, they develop insulin resistance and impaired glucose tolerance in addition to increased leptin levels compared to wild-type littermates (Nonogaki et al. 1998). The 5HT2CR mutation also enhanced the sensitivity of animals to high-fat diet induced obesity. Since the young mutant mice had normal plasma leptin levels and normal sensitivity to exogenous leptin administration, it was deduced that 5-HT_{2C} receptors are not required for leptin action and hyperphagia is not likely to result from perturbed leptin signaling in these mice. In other words, hyperphagia *per se* does not seem to alter leptin action in young animals. The 5HT2CR mutation primarily produces a dysregulation of food consumption rather than a deficit in the regulation of carbohydrate or fat metabolism. Hyperphagia leads to the development of late-onset obesity and insulin resistance. As young 5HT2CR$^{-/-}$ mice are not obese despite prolonged hyperphagia, this could indicate a presence of compensatory elevation of energy expenditure. Thus, "middle-aged" obesity in these mice could reflect an age-dependent decrement in the ability to compensate for elevated food consumption, and may lead to secondary decrease in leptin and insulin sensitivity (Nonogaki et al. 1998).

There is a common polymorphism in the human 5HT2CR gene, present in 10-20% of Caucasians, in which serine is substituted for cysteine in codon 23 (Lappalainen et al. 1995). Although this variation does not affect basal responses to 5-HT, the binding affinity of 5HT2CR agonist is altered (Malhotra et al. 1996). The role of the Cys23Ser substitution has been studied with regard to the development of human obesity as well as eating disorders (Burnet et al. 1999; Lentes et al. 1997), but no relation has been detected. The 5'-flanking region of the 5HT2CR contains regulatory regions and a putative transcription factor binding site (Shih et al. 1996). To find a polymorphism that could alter the expression level of the receptor this region was investigated in 123 obese (BMI $\geq$ 28 kg/m^2) and 466 non-obese (BMI < 28 kg/m^2) Japanese male patients of whom 138 had NIDDM (Yuan et al. 2000). Three loci of single nucleotide substitution (G$\rightarrow$A at –995, C$\rightarrow$T at –759, G$\rightarrow$C at –697) and a (GT)n dinucleotide repeat polymorphism at –1027 were identified. Of these polymorphisms, ten different haplotypes, five of which are rare, were constructed. The haplotype-based analysis showed a clear association of the polymorphisms with obesity (p=0.005). Haplotype "3" containing all of the substitutions was associated with leanness (p=0.02) and with glucose tolerance, i.e. it was more common in non-diabetic subjects (p=0.033). Haplotype "9" was associated with obesity (p=0.007). Furthermore, haplotype "3" showed increased promoter activity compared with the wild type. Thus, it was suggested that the nucleotide substitutions could be associated with the transcription level of the 5-HT_{2C} receptor gene and contribute to the genetic resistance to obesity and NIDDM (Yuan et al. 2000).

5.7 Rare congenital syndromes associated with obesity in humans

There are several Mendelian disorders listed in the OMIM (Online Mendelian Inheritance in Man) database, that exhibit obesity as one of their clinical manifestations. To date, twenty of these disorders have been mapped to a specific chromosomal location (Chagnon et al. 2000). Nine syndromes follow the autosomal dominant inheritance

pattern, six of them are inherited recessively in autosomes and five disorders are X-linked. The syndromes are listed in Table 4 with their chromosomal location and putative candidate genes, if known.

Table 4. Obesity-related Mendelian disorders with known map location. Modified from Chagnon et al. 2000.

Mode of inheri-tance	Syndrome	Candidate gene	Locus	Reference
Autosomal dominant	Achondroplasia (ACH)	FGFR3	4p16.3	(Shiang et al. 1994), (Superti-Furga et al. 1995)
	Albright Hereditary Osteodystrophy (AHO)	GNAS1	20q13.2	(Patten et al. 1990), (Schwindinger et al. 1994)
	Albright Hereditary Osteodystrophy 2 (AHO2)		15q	(Hedeland et al. 1992)
	Angelman Syndrome with Obesity (AGS)		15q11-q13	(Gillessen-Kaesbach et al. 1999)
	Posterior Polymorphous corneal Dystrophy (PPCD)		20q11	NA
	Insulin Resistance Syndromes (IRS)	INSR	19p13.3	(Wertheimer et al. 1994)
	Familial Partial Lipodystrophy Dunnigan (FPLD)		1q21-q22	(Kim et al. 1992) (Anderson et al. 1999)
	Ulnar-Mammary Schinzel Syndrome (UMS)	TBX3	12q23-24.1	(Bamshad et al. 1997)
	Thyroid Hormone Resistance Syndrome (THRS)	THRB	3p24.3	(Behr et al. 1997)
Autosomal recessive	Alström Syndrome (ALMS1)		2p13-p12	(Macari et al. 1998)
	Bardet-Biedl Syndrome 1 BBS1		11q13	NA
	Bardet-Biedl Syndrome 2 BBS2		16q21	NA
	Bardet-Biedl Syndrome 3 BBS3		3p13-p12	NA
	Bardet-Biedl Syndrome 4 BBS4	MYO9A	15q22.3-q23	(Gorman et al. 1999)
	Bardet-Biedl Syndrome 5 BBS5		2q31	(Young et al. 1999)
	Bardet-Biedl Syndrome 6 BBS6	MKKS	20p12	(Katsanis et al. 2000)
	Berardinelli-Seip Congenital Lipodystrophy (BSCL)		9q34	(Garg et al. 1999)
	Cohen Syndrome (COH1)		8q22-q23	NA
	Carbohydrate-Deficient Glycoprotein Syndrome Type IA (CDGS1A)	PMM2	16p13	(Matthijs et al. 1997)
	Fanconi-Bickel Syndrome (FBS)	SLC2A2	3q26.1-26.3	(Santer et al. 1997)
X-chromo-somal	Borjeson-Forssman-Lehmann Syndrome (BFLS)	FGF13	Xq26.3	(Gecz et al. 1999)
	Choroideremia with Deafness (CHOD)		Xq21.1-q21	NA
	Mehmo Syndrome (MEHMO)		Xp22.13-p21	NA
	Simpson-Golabi-Behmel 1 (SGBS1)		Xq26	(Pilia et al. 1996)
	Simpson-Golabi-Behmel 2 (SGBS2)		Xp22	(Brzustowicz et al. 1999)
	Wilson-Turner Syndrome (WTS)		Xp21.3-q22	NA

NA= not available

AIMS OF THE PRESENT STUDY

This study was carried out to investigate the genetic loci predisposing to obesity in Finnish sib pair material. In order to clarify the underlying genetic component of this multifactorial trait, three different strategies were applied: an analysis of candidate genes and candidate regions, a genome-wide screen of obesity, and finally, a quantitative trait locus analysis of body weight and height.

1. To investigate the role of biologically relevant candidate genes and genomic regions previously assigned in the animal models of obesity (I, II).

2. To search for new genetic loci predisposing to obesity by performing a genome-wide scan (III).

3. To identify loci influencing body weight and height by carrying out a quantitative trait locus analysis using genome scans of several study samples, mostly non-ascertained for obesity (IV).

MATERIALS AND METHODS

1. STUDY SUBJECTS

Study subjects were ascertained from two different sources for studies I, II and III, namely via the Helsinki University Central Hospital and the Finnish Twin cohort. For the study IV, several different sib pair study samples were combined.

1.1 Morbidly obese and control subjects in the association studies (I, III)

The morbidly obese study subjects had participated in the weight reduction program of the Helsinki University Central Hospital (HUCH) from 1989-1995. Altogether 420 invitation letters were sent to these patients, of which 254 (61%) patients were finally invited to the study examination. They all had had body-mass indexes (BMI) equal to or more than 40 kg/m^2 (at the time of the original recruitment their mean BMI was 45.3 ± 5.5 kg/m^2) and they were aged 21-64. The study group consisted of 184 women (72%) and 70 men (28%) with a mean age of 49 ± 9 years. Of these, 116 subjects (46%) were treated for hypertension and 59 (23%) subjects had type 2 diabetes. All the selected study subjects were weighed and their height and blood pressure were measured. A 12-hour fasting blood sample was drawn for DNA analysis and to measure serum lipid, glucose and insulin concentrations. All the study subjects were asked to fill out a questionnaire about their general medical history and medication, history of weight development and attempts to lose weight, exercise habits, consumption of stimulants, and the height and maximal weight of their first-degree relatives.

For the control group, 151 non-obese and apparently healthy subjects were ascertained from the same geographical area of Helsinki and its surroundings as the obese study subjects. The lean group consisted of 65 males and 86 females with the mean age 40 ± 11 years, and they all had BMI less or equal to 25 kg/m^2 (mean 22.3 ± 1.9 kg/m^2). All the subjects were unrelated and of Finnish origin. A venous blood sample was taken from all subjects for DNA analyses with the informed consent of study participants.

1.2 Affected sib and twin pairs and their family members (I, II, III)

A total of 105 sib pairs concordant for obesity from 92 families were ascertained through two different channels: firstly from the weight-reduction program of the HUCH (the one that was used to association analysis in study I) and secondly the Finnish Twin Cohort (Kaprio et al. 1978). The Finnish Twin Cohort consists of Finnish twin pairs (n=13888), born before 1958, including samples of same-sexed pairs, both monozygotic and dizygotic. Later, the Twin Cohort has been expanded also to include opposite-sex twin pairs (Silventoinen 2000). Surveys of the entire cohort have been carried out at regular intervals to collect longitudinal data on e.g. environmental and genetic risk factors (Kaprio 1994; Korkeila et al. 1991).

In the weight-reduction group (n=254), 58 probands having one or more obese siblings (BMI ≥ 32 kg/m^2) were selected for the sib pair study. A physical examination was conducted on the recruited sib pairs. Information about their medical history including

the development and stability of body weight, previous and present illnesses, use of medication, consumption of alcohol and cigarettes and exercise habits was obtained by interviewing the patients. The parents of the sibling pair were ascertained when possible, and a blood sample as well as a detailed health questionnaire was collected from them.

The additional 46 sibpairs from 34 families were ascertained from the dizygotic twin pairs, who replied in 1975, 1981 or 1990 to the mailed questionnaires of the Finnish Twin Cohort. These twins were same-sexed and born before 1958 (e.g. aged 38-67 at the time of this study). Each twin survey questionnaire contains items on weight and height, and those pairs in which both twins had had BMI $\geq$ 32 kg/m^2 were identified, and invited to participate in the study. A detailed health questionnaire was then sent to the selected twins, as well as their sibs and parents. The participating twin pairs were asked to give a blood sample for DNA analysis at their local health center, where their height and weight were also recorded.

Since it is important in a sib pair study to obtain maximum information for the phase determination, all the available parents, or an additional affected sibling, were collected. Both parents were available in 16 (17%) families and one parent was available in 24 (26%) families, and additional sibs were ascertained in 11 families (12%) leaving 45 sib pairs with no additional phase information. In ten of the 92 families there were three obese siblings in a sibship, and in two families there were four obese siblings. The remaining 80 families had two obese sibs in a sibship.

The characteristics of the sib pair material are given in Table 5.

1.2.1 Replication material (III)

To replicate the results of the genome scan (study III) in another sample set, an additional 93 affected sib pairs from 79 Finnish families were collected through three new weight-reduction groups. These patients had participated in the program at the Helsinki University Central Hospital, at the Tampere University Hospital and at Peijas Hospital in Vantaa. All the selected probands as well as their recruited siblings had BMI $\geq$ 30 kg/m^2. In 70 of these families, there were two affected siblings in a sibship, in six families there were three affected, in two families there were four affected and in one family there were five affected siblings. Parent(s) were available for three of those families that had only two affected sibs. A detailed questionnaire was mailed to all the selected subjects and they were asked to give a blood sample for DNA analysis at their local health center, where their height and weight were recorded. The characteristics of the replication material are given in Table 5.

Table 5. The characteristics of the two study groups, the primary genome scan sample set containing 100 sib pairs and the replication sample set containing 93 sib pairs.

	Height (cm)*	Weight (kg)*	BMI (kg/m^2)*	Females (n)	Males (n)
Primary sample set	166.3 ± 8.7	101.7 ± 20.2	36.7 ± 6.1	125	63
Replication sample set	170.0 ± 9.5	105.6 ± 20.9	36.5 ± 6.5	91	80

*The mean and standard deviation

1.3 Study samples for the combined QTL analysis (IV)

The subjects for the QTL analysis were derived from five different Finnish genome scans of complex traits: hypertension (Perola et al. 2000), obesity (study III), familial combined hyperlipidemia (FCHL) (Pajukanta et al. 1999), osteoarthritis (Leppävuori et al. 1999) and migraine (Wessman et al. 1998). Altogether, the study material consisted of 585 individuals from 252 families. Most of the selected families were originally from the Finnish Twin Cohort (Kaprio 1994), from which the concordant dizygotic twin pairs were ascertained according to their study criteria. In the case of FCHL, study families with evidence of dyslipidemia were chosen from the EUFAM study (Pajukanta et al. 1998). All the available parents and additional affected siblings were also collected. From the osteoarthritis study 94 individuals from 30 families were included in this combined study, from the obesity study 236 individuals from 87 families, from the migraine study 108 individuals from 47 families, from the hypertension study 139 individuals from 47 families and from the FCHL study 159 individuals from 41 families. The demographic details of all study groups are given in Table 6.

All the study subjects from the Finnish Twin Cohort Study had replied to the mailed health questionnaire of the Finnish Twin Cohort in 1981, 1990 or later, where they were asked among other questions about their weight and height. In addition to this, participants in the obesity and FCHL studies were clinically examined in the 1990's. The most recent weight, self-reported or measured, was used, the earliest record being from the year 1990.

Table 6. Demographic characteristics of the study groups in study IV.

	All	Obesity	Hypertension	Arthrosis	Migraine	FCHL
number of individuals	585	185	102	52	87	159
female/male	396/189	122/63	67/35	40/12	78/9	89/70
age[a] (SD)	50.9 (11.6)	49.9 (8.9)	56.7 (9.0)	58.6 (12.0)	42.6 (7.1)	50.3 (14.0)
range	20-85	21-72	39-74	20-80	32-62	22-85
BMI (SD) (kg/m^2) range	29.4 (6.8)	36.7 (6.1)	25.7 (3.1)	25.9 (4.2)	24.1 (3.8)	27.3 (3.9)
	17.4-56.6	22.3-56.6	18.6-33.5	17.4-39.9	17.4-37.9	17.4-37.2
skewness[b]	0.09	0.38	0.43	0.29	-0.25	0.73
kurtosis[b]	-0.39	0.37	0.29	0.53	-0.14	0.34
Height (SD) (cm) range	166.5 (8.5)	166.2 (7.7)	166.1 (8.8)	166.9 (7.7)	164.7 (5.8)	167.8 (9.4)
	144-194	144-194	150-186	155-186	151-182	147-191
skewness[b]	0.25	0.26	0.02	0.62	0.48	0.07
kurtosis[b]	-0.24	-0.14	-0.60	-0.07	0.38	-0.48

[a] individuals under 20 years of age were excluded from the phenotype analyses

[b] after corrections (1/BMI, square root of height)

2. DNA AND BIOCHEMICAL ANALYSIS

2.1 DNA analysis

2.1.1 Genotyping

DNA was extracted according to standard procedures (Sambrook et al. 1989). In studies I and II the subjects were genotyped using polymorphic markers within or flanking the candidate genes and the loci studied. Flanking markers were selected by consulting the Genome Data Base (at http://gdbwww.gdb.org). Radiation hybrid mapping (Cox et al. 1990) was carried out using the Stanford G3 Radiation Hybrid Panel (Research Genetics, Huntsville, AL) to clarify the interrelation of polymorphic markers and the MC4-R gene in the study II. In study III, in the primary genome scan, 374 microsatellite markers included in the Weber screening set version 6, spaced app. 10.0 cM apart and covering all 22 autosomes and X chromosome, were genotyped. The estimated average heterozygosity for the 374 markers was 0.76. In the second stage of the scan, 16 polymorphic markers were additionally analyzed for high-resolution mapping.

The PCRs were carried out with 15-25 μg of DNA in a reaction volume of 15-25 μl using an MJ Research thermal cycler. In studies I and II, one PCR primer of each pair was labeled in its 5' end by (γ-^{32}P) ATP or fluorescein isothiocyanate (FITC). The ^{32}P-

labeled PCR products were separated by polyacrylamide gel electrophoresis in 6% denaturing gels. Autoradiography was carried out on Kodak X-Omat films. The FITC-labeled PCR products were analyzed using an automated laser fluorescence (ALF) DNA-sequencing instrument (Pharmacia Biotech, Sweden). In study III, forward PCR primer of each pair was labeled with one of the three fluorescent dyes (FAM, HEX or TET) (Applied Biosystems, Foster City, CA) to enable detection. PCR products were separated on an ABI 377XL automated DNA sequencer (Perkin Elmer, Foster City, CA) using denaturing 6% polyacrylamide gel, with analysis by Applied Biosystems' Genescan 2.1 software (Perkin Elmer). Analysis and assignment of the marker alleles were done independently by two people with Genotyper 2.0 (Perkin Elmer).

2.1.2 Sequencing

In study III, the potential candidate gene on Xq24 encoding the 5-HT_{2C} receptor was examined more carefully by association analysis for a known polymorphism, a Cys23Ser amino acid variant and by sequencing. For assaying the 5-HT_{2C}R polymorphism, PCR amplification was carried out, and the PCR products were sequenced by cycle-sequencing using a BigDye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (Perkin Elmer, Foster City, CA, USA) on a model 377 automated DNA sequencer (Applied Biosystems). To confirm the sequencing results of three subjects, the Thermo Sequenase II dye terminator cycle sequencing premix kit was also used (Amersham Pharmacia Biotech, Ohio, USA).

2.1.3 Genotyping "in silico"

In study IV, the allele information of five genome scans were standardized by comparing the allele frequencies of each allele for each analyzed marker, and subsequently collapsing the alleles from individual scans to the same category based on its frequency. The reference numbering of alleles was derived from the obesity scan. The data was obtained from a total of 350 (SD±20) markers per scan.

2.2 Biochemical analysis

2.2.1 Leptin radioimmunoassay

In study I, leptin concentration was determined with radioimmunoassay (Considine et al. 1996), where the intra-assay coefficient of variation was 4.7% and interassay coefficient of variation was 6.2%.

2.2.2 Measurements of serum glucose, insulin and lipids

In the study I, serum glucose levels were determined by a commercial glucose dehydrogenase quantification technique (Banauch et al. 1975) and serum insulin levels were measured by a double-antibody radioimmunoassay (Desbuquois and Aurbach 1971) (Phadeseph Insulin RIA, Pharmacia, Uppsala, Sweden). The levels of total cholesterol, high-density lipoprotein cholesterol and triglycerides were measured using enzymatic techniques (Finley et al. 1978) (Boehringer Mannheim).

3. STATISTICAL ANALYSIS

The computer programs SAS (SAS Institute Inc., Cary, NC) and SYSSTAT (SPSS Inc., Chicago, IL) were used to determine sample means, variances and correlations. To test the equality of means of quantitative traits (leptin levels) by genotype (I), general linear modeling was used, and for variables that were distributed non-normally (BMI), the Kruskal-Wallis test was applied (III). The χ^2-test was used in association analyses to compare the frequencies of the different genotypes or categorical variables (I, III). The differences in markers allele frequency distribution between obese cases and lean controls were tested by the likelihood ratio test using the DISLAMB program (Terwilliger 1995) (I).

Prior to tests for linkage, Mendelian segregation was confirmed with PedCheck (O'Connell and Weeks 1998) (III) and MENDEL (Lange et al. 1988) (IV) programs. All the incompatibilities in genotypes were either resolved unambiguously or the offending families were discarded from all analyses. For affected sib-pair analyses in studies I, II and III, non-parametric allele-sharing methods were used to determine the identical-by-descent (IBD) status of the pairs. Two program packages were used, ANALYZE (Kuokkanen et al. 1996; Terwilliger and Göring 2000a) and MAPMAKER/SIBS (Kruglyak and Lander 1995) to perform two-point and multipoint analyses. The SIBPAIR program of ANALYZE package, performs pseudomarker linkage analysis. In sibships of more than two siblings, this algorithm does not break them into pairs, but rather analyzes them as sibships, which is generally more efficient. The statistic employed in the SIBPAIR program is computed as a lod score in which all parents are assumed to be informative for the disease, with all affected sibs inheriting the disease-predisposing allele from each parent. Estimates of marker allele frequencies were obtained from the pedigree data by taking the information from parents' genotypes (II) or from all individuals (III) using the DOWNFREQ (Göring and Terwilliger 2000c).

In study IV, our traits BMI and height were not normally distributed. Because of the non-normality, transformations to square root of height and 1/BMI were used in the analyses because they were found to represent best normal distribution in our sample. Age was set to represent the year of reported or measured weight or height when used as a covariant in the analyses. The linkage analyses were done using the Solar 1.625 program (Almasy and Blangero 1998; Blangero and Almasy 1997) and MAPMAKER/SIBS version 2.0 (Kruglyak and Lander 1995), with the ML variance option (Pratt et al. 2000) in the case of X-chromosomal markers. Since individuals with BMI $\geq$ 30.0 kg/m^2 originating from the obesity study and $\leq$ 27.0 kg/m^2 originating from the hypertension study were originally selected according to their BMI, they were labeled as probands in Solar analyses to ascertainment bias correction.

To evaluate the significance of our results, we permuted the phenotype values (age, sex, stature) randomly for 100 times and re-calculated the genome scans using these permuted phenotype values. In the permuted scans the same adjustments for covariates age and sex were used as in the original scan. We permuted rather phenotypes than genotypes because not all individuals were genotyped for all the markers.

RESULTS

1. ANALYSES OF CANDIDATE GENES AND REGIONS

1.1 Leptin

The leptin (*OB*) gene was the first candidate gene studied in this thesis, and its role in obesity was evaluated using different approaches. Genetic linkage and association were tested between obesity and polymorphic markers flanking the leptin gene (D7S686, D7S514, D7S635, D7S530, D7S680, D7S1875, HOB) in sib pair and case-control materials (I). No significant evidence for linkage to obesity was found in 76 concordant sib pairs (BMI ≥ 32 kg/m^2), neither was association found with 252 morbidly obese cases (BMI ≥ 40 kg/m^2) and 151 lean controls.

The correlations between serum leptin levels and BMI as well as marker alleles were studied in case-control material. A significant positive correlation was found in men (p=0.0001) and in women (p=0.0001) between leptin concentration and body-mass index (I). To date, this is quite an obvious result if we view leptin as hormone secreted by adipose tissue. Although there are individual variations, in principle, the more fat the more leptin is secreted in the circulation (Considine et al. 1996). When serum leptin levels were analyzed according to flanking markers, only the marker D7S530 was associated (p=0.04): more spesifically, allele 5 was associated with lower leptin levels (p=0.02) and allele 7 was associated with higher serum leptin levels (p=0.02). However, when the carrier status of these alleles was tested with BMI of the obese subjects, no association could be seen. Further, the leptin concentrations did not correlate with serum cholesterol, HDL-cholesterol or triglycerides, nor with serum glucose or insulin levels.

1.2 Other candidate genes

In study II, linkage between obesity and human homologues of seven murine obesity genes, one claimed “anti-obesity” murine gene and two loci, D2S1788 and D11S900, reported to be linked to obesity related phenotypes in restricted populations (Comuzzie et al. 1997; Norman et al. 1997), was tested. A total of 105 sib pairs were genotyped and analyzed for 30 polymorphic markers flanking the candidate regions. No significant evidence of linkage was detected in two-point analyses with any of the markers. However, several markers spanning a region of 14 cM, and lying in the immediate vicinity of the MC4-R gene, indicated excess sharing of the alleles IBD (Figure 5). Further, marker D11S1321 flanking UCP2 and UCP3 genes showed some evidence of increased allele-sharing (p=0.011) as well as marker D1S250 flanking the OB-R gene (p=0.054). Other markers in these chromosomal regions revealed no evidence for linkage. Two-point results of all the analyzed markers are given in Figure Y.

Based on the findings of two previously reported genome scans (Comuzzie et al. 1997; Norman et al. 1997), chromosomal regions 11q21-q22 and 2q21 were investigated, but no linkage between morbid obesity and the selected markers (D11S900, D2S1788) was found. However, we tested only for linkage with the final phenotype of obesity (BMI ≥ 32 kg/m^2) and not with specific quantitative phenotypes, such as % body fat or serum leptin levels.

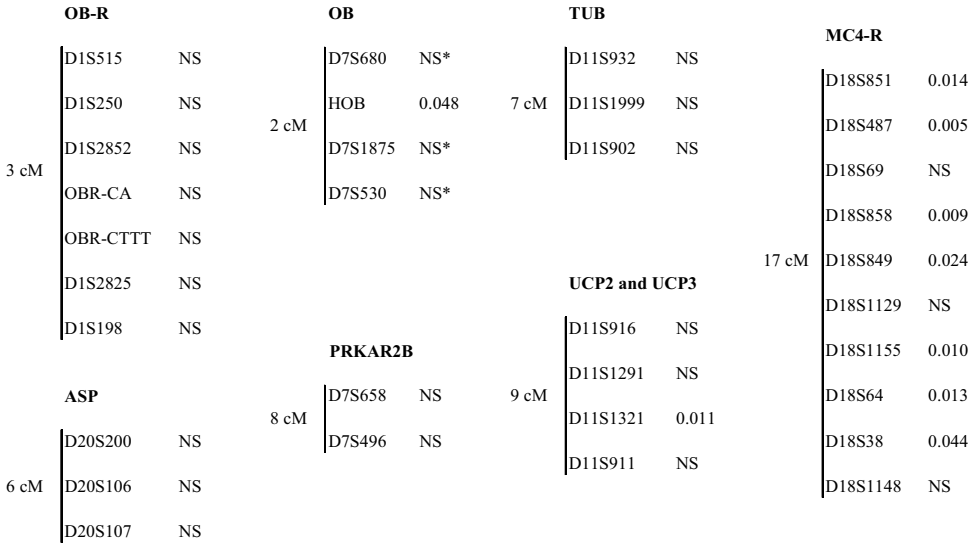

Figure 5. The p-values obtained from two-point linkage analyses between obesity and markers within and flanking the candidate genes in sib pair material. All the p-values over 0.05 were considered nonsignificant (NS) and were excluded from further analyses. The length of different regions is indicated by centimorgans (cM) (web sites: http://www-genome.wi.mit.edu and http://cedar.genetics.soton.ac.uk/public_html/index.html). *These markers flanking the OB gene were analyzed previously in study I.

To further increase genetic homogeneity and to dissect the clinical phenotypes, the study sample of 105 sib pairs was divided into five different subsets. The first and second subsets were composed of sib pairs having both parents originating either from the eastern or southwestern regions of Finland, respectively. The rationale for this was based on the well-demonstrated difference of risk factors for cardiovascular diseases between eastern and southwestern Finland (Vartiainen et al. 1994). The third group was selected to represent severe obesity having an inclusion criteria of BMI ≥ 37 kg/m^2 for all siblings in a sibship. In the fourth subgroup all diabetic individuals, defined on the basis of current medication for diabetes, were excluded. In the fifth subgroup only sib pairs having one lean (BMI ≤ 26 kg/m^2) and one obese (BMI ≥ 32 kg/m^2) parent were accepted. The number of sib pairs in these individual subsets, are given in Table 7.

Table 7. The p-values obtained from two-point analyses between obesity and markers of interest (D18S851, D18S487, D18S69, D18S858, D18S849, D18S1129, D18S1155, D18S64, D18S38) flanking the MC4-R gene and covering a region of 14 cM (http://www-genome.wi.mit.edu) in total material and different subgroups.

	N	S851	S487	S69	S858	S849	S1129	S1155	S64	S38
Total material	105	0.014	0.005	0.184	0.009	0.024	0.440	0.010	0.013	0.044
Parents originating from eastern Finland	38	0.051	0.048	0.159	0.022	0.062	0.500	0.092	0.373	0.239
Parents originating from western Finland	51	0.358	0.093	0.500	0.500	0.247	0.488	0.177	0.017	0.100
BMI ≥ 37 kg/m^2 for each sib, "severe" obesity	39	0.236	0.013	0.056	0.024	0.076	0.500	0.235	0.049	0.500
Sibs having medication for diabetes excluded	80	0.004	0.006	0.061	0.008	0.003	0.322	0.014	0.012	0.017
One parent having BMI ≤ 26 kg/m^2, the other BMI ≥ 32 kg/m^2	20	0.074	0.001	0.072	0.020	0.006	0.482	0.129	0.074	0.093

* N. means the number of sib pairs in each of the subgroups

Two-point analyses in different subgroups showed further evidence for linkage to markers D11S1321, D1S250 and markers flanking the MC4-R gene on chromosome 18. The subgroup from which all the diabetic subjects had been excluded revealed the strongest evidence for linkage between several MC4-R gene markers and obesity. However, the smallest p-value (p=0.001) was obtained with the marker D18S849 in a subset containing only sib pairs that had one lean and one obese parent (Table 2).

Being the most interesting candidate, the MC4-R gene was further examined by DNA sequencing. The coding, as well as 5'- and 3'- flanking regions were sequenced in seven obese subjects, belonging to the sib pair material. A single base substitution replacing, as judged from the published sequence (Mountjoy et al. 1994), valine (GTC) for isoleucine (ATC) at codon 103, was identified. All seven individuals were homozygous for the valine allele, and no carriers of the isoleucine allele were detected. Furthermore, at codon 169, where Mountjoy et al. (1994) reported sequence for serine (AGC), all the cases had a sequence corresponding to isoleucine (ATC). Also, one additional adenine nucleotide was found in the 3'-flanking region in all the obese subjects between nucleotides 1114 (A) and 1115 (T). All these sequence variations found in Finnish obese subjects had been previously identified in white British males (Gotoda 1997).

1.3 Three stage genome-wide scan

To search for novel loci predisposing to obesity in the Finnish population, a genome-wide screening was performed (III). At this time, five previous obesity scans had been published from other populations (Comuzzie et al. 1997; Hager et al. 1998; Hanson et al. 1998; Lee et al. 1999; Norman et al. 1997). Therefore, it was of interest also to see, whether those findings could be replicated in the genetically isolated Finnish population.

The genome scan of obesity (defined as a BMI ≥ 32 kg/m^2) was carried out in three stages with initially 100 sib pairs (altogether 188 affected) from 87 families. In the first stage, two-point analyses of the 374 scan markers revealed nine regions with marginal evidence for linkage (MLS >0.8) on chromosomes 1q, 4q, 5p, 9p, 10q, 12q, 16q, 18q and Xq (Table 8). Generally, the multipoint ML scores tended to be lower than the values for two-point MLS. The most significant evidence for linkage was found on chromosome Xq, with the marker DXS8064 giving a two-point MLS of 3.14. The highest multipoint values were established on chromosomes 18q and Xq, where the MLS reached 2.16 and 2.10, respectively. In addition, multipoint MLSs >1.0 were found on chromosomes 1, 5, 10, 11 and 12.

In the second stage, to increase the phase information, 16 screening set markers were genotyped on the positive regions showing two-point MLS >0.8, with all the available parents. This resulted in an increase of ML scores on chromosomes 1q, 4q, 12q and 16q, whereas the ML scores decreased or did not change on chromosomes 5p, 9p, 10q and Xq (presented in Table 8).

Table 8. Single-point maximum likelihood scores (MLS) of markers revealing marginal evidence for linkage, MLS of markers after genotyping the parents, parametric LOD scores and markers chosen for saturation mapping are indicated (*data also published in Study II).

Marker	MLS > 0.8	MLS with parents genotyped	Parametric LOD scores	Saturation mapping
D1S518	1.08	1.44	1.37	x
D4S2368	1.19	1.37	0.62	x
D5S1470	1.19	1.07	1.00	x
D5S1501	1.04	0.74	0.75	x
D5S1462	0.87		0.87	
D5S1456	1.17	1.11	0.76	x
D9S921	0.93		0.00	
D9S1118	1.18	1.16	0.73	
D9S158	1.01	0.87	0.64	
D10S1223	0.93		1.06	
D10S169	1.25	0.89	0.73	
D11S2002	0.86		0.35	
D12S2070	1.08	1.17	0.46	x
D12S395	1.26	1.32	1.86	x
D16S3110	0.84	1.26	1.01	
D18S851	1.62		0.86	x
D18S487*		1.97	1.87	
D18S858	0.83		0.98	
D18S1155*		1.82	0.70	
D18S64	1.61	1.59	0.98	x
D18S38*		0.80	0.43	
DXS6800	0.85	0.76	0.50	
DXS6799	1.20	1.16	0.36	x
DXS6804	**3.14**	2.96	1.60	x
DXS1001	1.21	1.41	0.00	x

For the fine-mapping, we selected six regions on chromosomes 1q, 4q, 5p, 12q, 18q and Xq that provided two-point MLS >1.0. We performed genotyping using 24 additional markers on these regions in our initial study material of 188 individuals (100 sib pairs). The saturation mapping supported evidence for linkage only on chromosomes 18q and Xq (Table 9). On the other regions, all the obtained ML scores remained <1.0.

For the third stage of the scan, a replication material of 171 obese (BMI ≥ 30 kg/m^2) individuals (93 sib pairs) from 79 families, were ascertained. Selecting the markers with the best evidence for linkage, five markers on chromosome 18q21, and five markers on

Xq24 were genotyped. Allelic data for the replication sample set was analyzed separately as well as in pooled analysis with the original study material. The replication material alone provided additional evidence for linkage on the Xq24 region as the two-point MLS of DXS8067 was increased from 0.6 to 2.5. The two-point MLS for other reanalyzed markers did not change significantly. In the pooled analysis of 193 sib pairs, two-point ML scores further increased on the Xq24 region: the MLS of DXS8067 increased to 2.7 and that of DXS1001 to 2.2 (Table 4), and the multipoint MLS reached a significant value of 3.5 (Fig. 6).

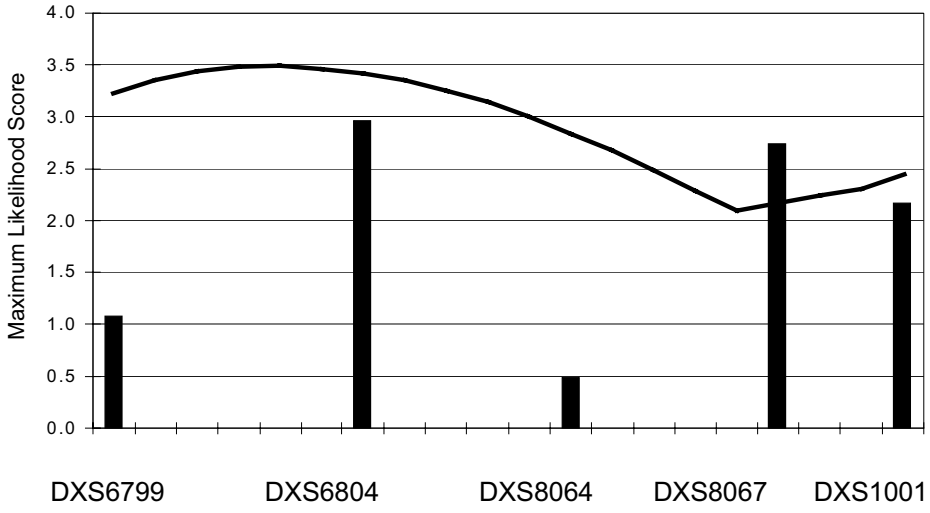

Figure 6. Two-point (represented as bands) and multipoint (line) ML scores over the Xq24 region in the extended material in the third stage of the genome scan.

None of the 18q region markers showed strong evidence for linkage in the replication material, the highest two-point MLS being 0.9 with marker D18S487. However, the pooled analysis including 193 sib pairs strengthened the linkage to 18q2: the two-point MLS of D18S1155 increased from 1.8 to 2.4 and that of D18S64 from 1.6 to 2.3 (Table 9). The multipoint MLS values did not exceed the significance level in this genomic region.

Table 9. Data from three stages of the genome scan. Two-point ML scores obtained with the markers on chromosomes 18q21 and Xq24 providing the strongest evidence for linkage.

Marker	Initial genome scan	Fine-mapping	Extended study material
DXS6799	1.20	1.16	1.08
DXS6804	3.14	2.96	2.96
DXS8064		0.96	0.49
DXS8067		0.63	2.73
DXS1001	1.21	1.14	2.17
D18S851	1.62	1.39	0.29
D18S487		1.97	1.84
D18S858	0.92	0.92	0.27
D18S1155		1.82	2.42
D18S64	1.61	1.59	2.32

1.3.1 Serotonin receptor 2C

On the Xq24 region, the most potential candidate gene, 5-HT_{2C} receptor (Nonogaki et al. 1998), was selected to be examined more carefully by association analysis and DNA sequencing. A previously reported polymorphism of this gene, a Cys23Ser amino acid variant (Lappalainen et al. 1995) was examined to test for differences in allelic frequencies conditional on phenotype. This polymorphism was genotyped in an association material of 254 morbidly obese subjects and 134 lean controls. The genotype and allelic frequencies did not differ between the study subjects and the controls (p=0.42 for females, p=0.96 for males).

To further study the 5-HT_{2C}R gene for sequence changes, 18 obese males and 19 lean male controls were screened. The coding region and the known promoter region of 7.3 kb (Xie et al. 1996) were sequenced, but no DNA-variants correlating with obesity were found. In exon 3, a single-nucleotide polymorphism (C→A) -42 bp upstream of the 5'-end of the coding region was identified in one obese male and the previously published (G→C) polymorphism in codon 23 (Cys23Ser) was detected in another case. In the promoter region, two polymorphisms that were present in three cases and six controls were found: a C→T variation at position -757 and a G→C variation at position -690. In addition, we found two single-nucleotide insertions in all cases and all the controls: compared to the published sequence, there was one additional guanine (G) in position -815 and another G in position -870 upstream of the 5'-end of exon 1.

1.4 QTL analysis of body-mass index and stature

In study IV, analyses were carried out on total study material as well as on males and females only. Since the sex-specific results did not differ significantly from the results of the total study sample, only the age- and sex adjusted results were reported. Further, only

the multipoint results were given with no two-point lod scores, since not all of the markers were analyzed in all of the scans.

1.4.1 Body-mass index

The heritability of BMI in the study sample was found to be 0.49. Sex ($p=0.03$) and age $\times$ sex ($p=0.01$) were significantly correlated with BMI and thus were included in the final model. The highest multipoint lod score for BMI was found in chromosome 5, peaking at 1.54, 54 cM from p-ter. No other chromosome showed lod scores ≥ 1 for BMI in the multipoint analysis.

1.4.2 Stature

The heritability of stature in total study sample was found to be 0.70. This corresponds well to a recent study of heritability of height in the Finnish population, where heritability was 0.77 (95% CI=0.73, 0.82) among men and 0.76 (95% CI=0.71, 0.80) among women (Silventoinen et al. 2000). Sex ($p<0.0000001$) and age ($p=0.04$) were significantly correlated with stature and thus they were included in the final model. The highest multipoint lod score was recorded in chromosome 18q, peaking at 3.01, 74 cM from the pter at the location of marker D18S858 flanking the MC4-R gene (Figure 7). Also, in chromosome 7 a lod score of 2.82 was found 163-164 cM from pter. The only other chromosome with a lod score over 2 was 12, where a lod score of 2.26 was obtained at 65 cM from pter. No clear candidate genes influencing stature have yet been located in the regions of chromosome 7 and 12, although both of the regions are quite wide. In the multipoint analyses, no other chromosomes showed any significant evidence for linkage to stature.

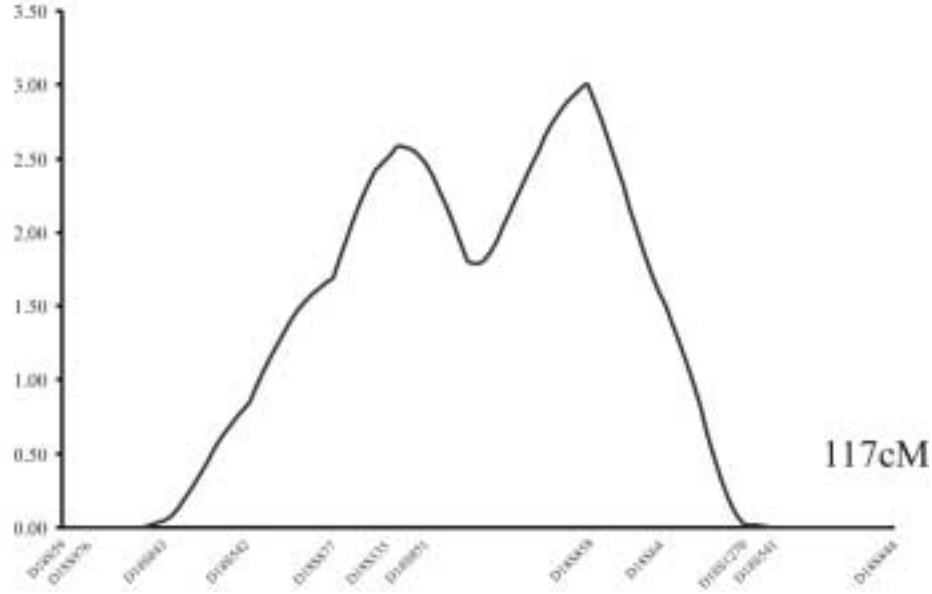

Figure 7. The multipoint lod score curve on chromosome 18 in the QTL analysis of stature. The highest lod score peaks at 3.01. The used chromosome 18 markers are shown on the x-axis.

To assess the statistical significance of our results, we performed simulations. In the 100 permutated genome scans, we found the multipoint lod score > 3.01 a total of five times, giving an empiric p-value 0.05. Lod scores of over 2.82 were seen seven times (p=0.07) and 2.26 thirty-six times (p=0.36). No permuted genome scan showed a combination of three or more lod scores > 2.26 (p<0.01).

DISCUSSION

1. PHENOTYPE OF OBESITY – OBESE STUDY SUBJECTS

In genetic studies of complex diseases, fairly large sample sizes are needed to detect common alleles of slightly modifying effects from the "noise" of environment. Enough power is essential even if genetically isolated populations are used (Terwilliger et al. 1998). Additionally, careful dissection of phenotype and subgrouping of the sample set give a better chance to detect several loci of a small effect than a group of study subjects robustly tied together with loose criteria presenting the same diagnosis. In fact, most of the complex diseases could probably be allocated to several subclasses or variables of the "mother disease": for instance, in case of schizophrenia or migraine, patients with the same diagnosis do not necessarily have the same symptoms, and according to the clinical picture, they represent several subclasses of the disease. In quantitative traits, categorization can be made by setting cut-points to the continuous variable, e.g. mild or severe hypertension based on the selected blood pressure values.

In genetic studies of obesity, a tendency towards refining the phenotype has been clear. To dissect the phenotypic picture of obesity, much more should be done than just obtaining a certain cut-off point of body-mass index to measure morbid obesity. Several aspects have been taken into account, e.g. behavioral (binge eating), age-of-onset (childhood obesity), related diseases (NIDDM, endocrinological derangement), proportions of fat-mass and fat-free mass, respiratory quotient (to measure basal metabolic rate), plasma levels of secreted proteins (leptin), physical activity and the waist-to-hip ratio (Bouchard et al. 1988; Chagnon et al. 1997; Comuzzie et al. 1997). The genetic background has also been utilized to homogenize the study group (Norman et al. 1997; Rotimi et al. 1999).

In this study (I, II, III) the phenotype of obesity was based solely on the selected BMI cut-off point: the first recruited probands from the weight reduction group, the morbidly obese subjects, had had BMI $\geq$ 40 kg/m^2, and their ascertained siblings BMI $\geq$ 32 kg/m^2. For the replication sample set (III) the criteria was BMI $\geq$ 30 kg/m^2. The leptin levels were measured only from the morbidly obese cases (n=252) from the weight reduction group (I). Presumably, it would have been advantageous to refine the phenotype by measures of, for instance, body composition or basal metabolic rate, since BMI provides only an indirect measure of fatness and is not the most accurate indicator of obesity (Borecki et al. 1991). However, the morbidly obese cases represent the extreme end of the phenotype, and could be useful to increase the heritable component of the disease (Lander and Kruglyak 1995). Approximately half of the recruited sib pairs were dizygotic (DZ) twins ascertained from the Finnish Twin Cohort. DZ twin pairs represent ideal study material for sibling pair analyses, for they are perfectly matched for age and closely matched for many pre- and post-natal environmental factors (Martin et al. 1997). The size of the sib pair study sample was limited, when compared to other genetic studies of obesity (Chagnon et al. 1997; Comuzzie et al. 2000; Norman et al. 1996). However, the advantage of using the homogeneous and genetically isolated Finnish population has been proved by excellent examples of identification of loci predisposing to complex diseases, even with small sample sizes (Kainulainen et al. 1999; Kuokkanen

et al. 1996; Leppävuori et al. 1999; Perola et al. 2000).

In study II, a subgrouping of the sample set, based on different perspectives, was used. First, the geographical origin of the proband's parents was implied – the rationale for this was derived from the well-demonstrated difference in risk factors for cardiovascular diseases between eastern and southwestern Finland (Kittles et al. 1998; Vartiainen et al. 1994). Second, only pairs where both represented severe obesity (BMI ≥ 37 kg/m^2) were included. Third, the idea was to exclude diabetic subjects, and thus remove confounding factors such as co-morbidities. The last group was reasoned to represent a clearer mode of inheritance, i.e. predisposing allele descending from one side only: thus, only those pairs, where one parent was obese (BMI ≥ 32 kg/m^2) and the other lean (BMI ≤ 26 kg/m^2), were selected. The division of the study sample into subclasses was found to be advantageous, since more significant p-values were obtained with the MC4-R flanking markers in the group of non-diabetic (p=0.003) and in the group that imitated the "dominant inheritance model" (p=0.001).

For the sequencing analysis of MC4-R and 5HT2CR (II, III) only a few study subjects were used. To search for sequence variations in MC4-R, nine obese cases were screened, and for 5HT2CR, 19 cases and 18 controls were analyzed. Recently, it has been suggested that among the morbidly obese subjects, 3-4% carry an obesity causing mutation in their MC4-R gene (Vaisse et al. 2000), and thus far, these would represent the most frequent mutations causing dominantly inherited human obesity. In view of this, it is no wonder, that our study failed to demonstrate any obesity associated DNA variants from the tiny sample set: to catch one mutation among these seven cases, the probability would be around 25%. However, we did find DNA variants that had been previously identified in Caucasian populations (Gotoda et al. 1997) in the MC4-R gene, but evidently these were conservative polymorphisms not altering the coding region. Sequencing of the 5HT2CR revealed a previously published amino acid variation (Lappalainen et al. 1995), but this was not associated with obesity in our study, although it has been implicated in eating disorders (Burnet et al. 1999). However, in our analysis we used only the simple phenotype of obesity, any behavioral aspects concerning the aetiology of obesity were not considered.

In study IV, the quantitative trait loci of body-mass index and height were searched for in the Finnish population by combined analysis of five genome scans. Using the 1/BMI and the square root of height as variants normalized the distribution of these continuous phenotypes. Convincing linkage to two loci affecting stature were identified in our study material, but no linkage was detected to BMI. This may reflect the difference between these two continuous variables, e.g. the extent of confounding factors affecting their outcome. The height of adults is usually attained during the puberty, and it remains essentially the same throughout life, whereas BMI may vary considerably during an individual's lifetime. In other words, there are far more environmental factors affecting BMI than stature in adult age, and one occasional measurement of weight at one occasional moment of time is naturally less characteristic for a person than his or her height. Further, as it has been shown, the use of extreme concordant or discordant pairs could yield much more significant results in QTL analysis than the use of such pairs that represent both mean values of a continuous variable (Risch and Zhang 1995). So far, no studies have been reported, where QTL affecting body weight has been found with normal weight human subjects.

Furthermore, in our study material, the heritability of stature was higher than that of the BMI (0.70 vs. 0.49). This may, in part, explain why linkage was detected for stature but not for BMI. It has been reported, that extreme obesity is more heritable than moderate overweight, i.e. the familial risk ratio for extreme obesity is higher than for moderate levels of obesity (Lee et al. 1997). Thus, the number of families or relative pairs required to achieve adequate statistical power in gene mapping studies of obesity can be reduced substantially by focusing on family members of extremely obese individuals (BMI ≥ 40 kg/m^2). Furthermore, Allison et al. (Allison et al. 1996) investigated the λ statistics in obesity, and concluded that λs for milder forms of obesity were disappointingly low. Therefore, an impractically large number of sib pairs would be necessary to reach a sufficient power to detect linkage for obesity related genes. Since our study population consisted mainly of normal weight subjects, the current methods as well as the size of the study sample may prove insufficient to detect a locus affecting the relative body weight, i.e. BMI, especially if this sort of locus has only a minor genetic effect.

2. LINKAGE TO OBESITY - SUGGESTIVE OR SIGNIFICANT

In study I, the marker D7S530 was found to be associated with variations of serum leptin levels in morbidly obese subjects. This particular marker showed the strongest evidence for linkage to obesity in a study of French sib pairs (Clement et al. 1996), but in our study (I, II), no association or linkage between D7S530 and obesity *per se* could be seen. Based on the most recent genome scan for loci linked to plasma leptin levels, it has been suggested that the genetic determinants of leptin concentration and BMI are, to some extent, distinct (Walder et al. 2000), because no chromosomal region demonstrated evidence of linkage with both BMI and plasma leptin levels. Although several markers flanking the *ob* gene have shown suggestive linkage to obesity in some populations (Clement et al. 1996; Reed et al. 1996), contradictory data has been presented by others (Hasstedt et al. 1997; Norman et al. 1996; Stirling et al. 1995). Our study showed no evidence for linkage, or association of any *ob* gene markers to obesity in the Finnish population (I, II). Our finding is in agreement with the view that obesity-causing mutations in the *ob* gene are extremely rare in human subjects (Considine et al. 1995; Maffei et al. 1996).

According to the Human Obesity Gene Map, an extensive annual review gathering data from all reports concerning the genetic studies of obesity (Chagnon et al. 2000), putative loci affecting obesity-related phenotypes are found on all but the Y chromosome of the human chromosomes. The number of genes and other markers associated or linked with human obesity phenotypes continues to grow and is now well over 200. Results of linkage and association studies of human homologues of murine obesity genes from different populations have been conflicting. The reasons for this could include different ethnic backgrounds, criteria for patient selection and the variety of phenotypes analyzed. Eventually, some of these findings will turn out be to false-positives whereas some of them will certainly prove to be replicable and thus more important than others.

Initially, no evidence for significant linkage to any candidate gene was detected in study II. The sample size of our sib pair material was initially quite small and divisions to subsets further decreased it (II). In non-parametric linkage analysis, very restricted sample sizes lead to the power of the study to decrease, and also to an increased chance

of type 1 errors. However, given the p-values were not highly significant, excess IBD sharing was observed in elongated, 14 cM region flanking the MC4-R. A study which shows that relatively wide peaks could represent a reliable linkage compared to narrower peaks (Terwilliger et al. 1997), strengthens our finding of suggestive linkage to MC4-R. Considering the MC4-R finding in the genome-wide context (III), further support for the result of our candidate gene study may be derived. In the genome screen, the second most significant evidence for linkage came from markers flanking the MC4-R gene, i.e., a two-point maximum-likelihood score of 2.42 for marker D18S1155 in the extended sib pair material. The significance level of this MLS corresponds relatively well to our best p-values in the candidate gene analysis (p=0.001). Looking retrospectively, it is of great interest, that the subset of sib pairs representing "dominantly inherited obesity" (one lean parent and one obese parent), gave the most significant p-value in study II. At the time of this study, it was not known that the MC4-R mutations cause dominantly inherited human obesity, nor was it proposed that these mutations are such a frequent (3-4%) contributor to human morbid obesity (Farooqi et al. 2000; Vaisse et al. 2000).

3. GENOME SCANNING AND BEYOND

In genome-wide searches completed in other populations, evidence for linkage of different obesity-related phenotypes has been reported to several loci on different chromosomes (Table 10) (Comuzzie et al. 1997; Hager et al. 1998; Hanson et al. 1998; Lee et al. 1999; Norman et al. 1997; Walder et al. 2000). None of the earlier screens have reported a significant linkage to the X-chromosome. However, supportive data for our finding was derived from the genome screen of French obese sib pairs (Hager et al. 1998) where some evidence for linkage was reported to the same chromosomal region Xq24 (multipoint MLS 1.42 with DXS1001). Additionally, they found a suggestive linkage to Xp (multipoint MLS of 2.42 with DXS1226). Even if we did not detect significant linkage to the same regions as the previous genome scans, our results do not exclude any earlier findings: those loci just do not seem to be the major determinants of obesity in the Finnish population. The detection of loci with modest effects would most probably require a much larger sample size than was available in our study (Allison et al. 1996). The inconsistent findings across the different scans certainly reflect differences in ascertainment of study subjects, definition of phenotypes as well as variability in genetic background.

Table 10. The most significant quantitative trait loci, the nearest markers and the most significant lod scores obtained in genome scans of obesity and related traits in other populations. Modified from Chagnon et al. (2000).

Marker	**Location**	**Phenotype***	***N* sib pairs**	**Lod score**	**Ref.**
D1S550	1p31-p21	24 h RQ	236	2.0	Norman 98
D2S1788	2p21	Leptin, Fat mass	>5000 relative pairs	4.5 2.8	Comuzzie 97
D2S165, D2S367	2p22-p21	Leptin	264	2.4 / 2.7	Hager 98
D5S426	5p11	Leptin	264	2.9	Hager 98
D6S271	6p12-21	Leptin	1199	2.1	Walder 2000
D8S1110	8q11.1	Leptin	>5000 relative pairs	2.2	Comuzzie 97
D10S197	10p12.3	Obesity	264	4.9	Hager 98
D11S2000, D11S2366	11q21-q22	%fat	277	2.8	Norman 97
D11S2366	11q22	%fat	451	2.1	Norman 98
D11S976	11q23-q24	24h EE	236	2.0	Norman 98
D11S912	11q24	BMI	1766	3.6	Hanson 98
D18S877	18q21	%fat, FFM	451	2.3	Norman 98
D20S107, D20S211, D20S149	20q13	BMI>30, % fat	423	3.0< Lod <3.2	Lee 99
D20S601	20q11.2	24h RQ	236	3.0	Norman 98

* EE= energy expenditure; F = skinfold; FFM = fat free mass; RQ=respiratory quotient; BMI=body mass index; RMR=resting metabolic rate; WHR=Waist to hip circumferences ratio.

Pertaining to our results, there was some overlap in positive regions with genome-scan by Lee et al. (1999): on chromosome 1, our marker D1S518 (location 204 cM from p-ter) giving two-point MLS 1.4 corresponds to their D1S194 (206 cM) with a p-value of 0.0126 for percentage of fat (%fat). This region on 1q21-23 is homologous with pig and mouse obesity QTLs (Andersson et al. 1994; Taylor and Phillips 1996). On chromosome 9, our D9S158 (158 cM) with two-point MLS 1.0 is near their D9S1863 (151 cM) with a p-value of 0.185 for BMI. On chromosome 10, our D10S1223 (160 cM) and D10S169 (171 cM) with two-point MLSs 0.9 and 1.3 respectively, are in concordance with their D10S587 (170 cM) with a p-value of 0.0113 to %fat.

On the MC4-R region on 18q, our results in the genome-wide scan support the positive findings of our candidate gene study (II) showing the same level of significance. A novel important finding is that our results suggest that a major locus affecting the obesity phenotype lies on chromosome Xq24. An X-chromosomal obesity gene would be consistent with the fact of sex-specific effects on BMI: sex differences exist between obesity phenotypes, for instance abdominal obesity is more prevalent in males (Dionne et al. 1999; Kissebah and Krakower 1994), and it has been indicated in twin studies that genes contributing to the variation in BMI are not identical for men and women (Harris et al. 1995; Pietiläinen et al. 1999). Our interest on the Xq24 region focused on a

candidate gene, serotonin receptor -2C (5HT2CR), which had been widely implicated in body weight regulation in humans (Heisler et al. 1998; Leibowitz and Alexander 1998; Sargent et al. 1997) and in mice (Nonogaki et al. 1998). In addition, several other functionally relevant candidate genes have been assigned to our region of interest, for example the fibroblast growth factor homologous factor 2 (FHF2) (Gecz et al. 1999), the NDUFA1 gene, which is a component of NADH:ubiquinone oxidoreductase in mitochondrial complex I (Zhuchenko et al. 1996), and the brain mitochondrial carrier protein-1 (BMCP1) (Sanchis et al. 1998).

The involvement of 5HT2CR was investigated first with an association analysis, which was performed with a known amino acid substitution (Lappalainen et al. 1995), and secondly, by sequencing the coding and promoter region of 7.3 kb. No association or obesity-associated DNA-variants were found. The results are in agreement with a similar study (Lentes et al. 1997) which found no evidence for association of this polymorphism with obesity. However, it could have been revealing to sequence the promotor region with a larger set of samples, since a Japanese study found several nucleotide substitutions in the 5'-promoter region (Yuan et al. 2000). The constructed haplotypes were associated with either obesity or non-obese non-diabetic status.

In study IV, a combined QTL analysis of five genome scans was performed to identify new loci affecting body weight. By pooling raw data from multiple data sets, our study represents a novel aspect in meta-analytic approaches. In epidemiology, conventional approach is to rather pool summary statistics than genotype data (Allison and Heo 1998b; Gu et al. 1999b; Li and Rao 1996).

In addition to BMI, we selected body height as a continuous variable, since it affects the value of relative body-mass. Further, with the study of Hirschhorn et al. (Hirschhorn et al. 2001 submitted) this study represents the first reported genome-wide scan of stature. A suggestive linkage between stature and chromosome 18q was found. The highest multipoint lod score (3.01) was obtained with marker D18S858 flanking the melanocortin –4 receptor gene (MC4-R). It is of interest, that in addition to being obese, the human subjects with MC4-R mutations are also tall and have an increased bone mineral density (Farooqi et al. 2000; Yeo et al. 2000). In other species such as pigs and mice, the MC4-R mutations affect growth and increase body length (Huszar et al. 1997; Kim et al. 2000). Since both obesity and short stature have been shown to be risk factors for coronary heart disease (Eckel 1997; Forsen et al. 2000), this raises a tantalizing question whether MC4-R could be a susceptibility gene behind these two risk factors. Furthermore, an earlier genome scan of Finnish families with early-onset coronary heart disease reported evidence of linkage to a marker GATA26C03 only 20 cM from our area, the multipoint lod score peaking at 1.68 and two-point lod score peaking at 2.12 (Pajukanta et al. 2000). As the multipoint lod score method does not necessarily peak at the exact spot of the underlying disease gene (Kruglyak and Lander 1995), it is not impossible that a gene in this region could be a candidate for all these traits.

The second piece of suggestive evidence for linkage to body height was found at chromosome 7qter with a multipoint lod score of 2.82. The relevance of this finding is emphasized by the results of Hirschhorn et al. (Hirschhorn et al. 2001 submitted) replicating this very same region in their Swedish population (multipoint LOD = 3.40). Further, their Finnish sample set from the population of Helsinki shows some additional evidence of linkage with a lod score of 1.7 close to the qter of chromosome 7. Yet

another overlapping region between these two studies is on chromosome 12q, where Hirschhorn et al. report evidence for linkage (multipoint LOD = 3.35) with their Finnish subgroup from Helsinki in the very same region as us. Since both chromosomal intervals on 7qter and 12q are quite wide, no clear positional candidate genes can be speculated on.

Our novel meta-analytic approach of combining genome scans produced convincing results in the QTL analysis of stature, but the results concerning BMI remained very modest. However, this work should be taken as a proof-of-principle of new possibilities arising from the use of the enormous amount of data generated by several genome scan studies. In a pooled meta-analysis of multiple data sets one could choose between pooling of raw data or summary statistics. The putative problems of this novel approach include the ascertainment bias of study material, the heterogeneity of sample sets and ethical issues. Combining subjects that are originally selected on the basis of very different criteria may represent a too heterogeneous sample set. Thus, certain phenotypic characteristics might be unsuitable for a QTL study, for which samples of the extreme ends of trait may be the most advantageous ones (Risch and Zhang 1995). Ethical aspects should not be neglected when analyzing such phenotypic or clinical data that were not originally intended to be used for such research. An informed consent of participants is naturally required for each additional study apart from the original one, and lack of the consent would most probably be the greatest problem hindering the use of this novel approach. However, if this is taken care of, using all the collected data is highly justified from both economical and ecological point of views.

CONCLUDING REMARKS AND FUTURE PROSPECTS

Ever since the genetic studies of single gene Mendelian disorders have become undemanding and quite simple methodologically, the studies of complex diseases have attracted the increasing interest of geneticists. The rate in determining disease genes will certainly be accelarated due to the publication of the human genome sequence (Consortium 2001; Venter et al. 2001). Today, more and more studies of the common multifactorial traits are carried out, in attemps to find genes and alleles that contribute to the development of polygenic and environmentally influenced phenotype. The design of new technical tools to advance these large-scale studies has been so rapid that the development of new statistical tools has been following one step behind. This trend seems to continue as the society of geneticists is eagerly moving from the "old" linkage studies with microsatellite markers to the "new" linkage disequilibrium studies with single-nucleotide polymorphisms (SNPs). Multiplex expression array studies will also provide new implements in studies of complex diseases. One of the most powerful methods to investigate complex diseases is animal models. They provide enormous amounts of important new knowledge of disease pathogenesis, development of phenotypic picture and the role of putative candidate genes in a particular disorder. The "purification" of the genetic background is simpler with animal strains and thus, subtle effects are seen more easily in animals than in human subjects.

Today, the task of finding common allelic variants of marginal genetic effect on phenotype is very demanding work requiring a lot of resources. The enthusiastic belief that the minor effects of susceptibility alleles would account for a substantial proportion of the population risk for common diseases in a clear predictive way, may turn out to be largely exaggerated (Weiss and Terwilliger 2000). The proportions of the environmental impact and heritability are still hard to assess, although twin and adoption studies have offered some estimates. It remains to be seen on which scale all the collected knowledge of genetic variations in complex traits will improve the diagnosis, treatment and prevention of common diseases, and how widely new pharmaceutical substances based on the susceptibility genes could be used in public health care.

In genetic studies of obesity the discovery of leptin in 1994 (Zhang et al. 1994) had a tremendous effect on research field. Ever since our cognizance of the mechanisms of body weight regulation, adiposity and adipocyte metabolism as well as feeding behavior have increased dramatically. The interplay and concerted action of hypothalamic neurotransmitters have been elucidated with animal models and the hunt for yet unraveled missing links continues vigorously. The genes responsible for severe human obesity are finally starting to be elucidated, but a lot of hard work, time and good luck are still needed before most of the susceptibility genes and predisposing alleles for the common form of human obesity are incontestably resolved.

This thesis in its own tiny part, found significant evidence of putative genetic loci influencing obese phenotype, and thus provides interesting pieces for the huge ambiguous puzzle of this complex trait. This thesis also offers evidence of genetic loci influencing another aspect of growth, i.e. body height. Yet, further studies are needed to determine the underlying genes and alleles and their definite roles in the regulation of body composition.

ACKNOWLEDGEMENTS

This study was carried out in the Department of Human Molecular Genetics in the National Public Health Institute. I wish to express my gratitude to the director of the Institute, Prof. Jussi Huttunen, for providing excellent research facilities.

I wish to thank Prof. Leena Peltonen (Palotie) for being my supervisor. This thesis work would never have begun without her stylish lecture in Medical School during the first year of my studies – ever since her brilliant showmanship has not failed. Her wide knowledge in the field of molecular genetics and passion towards her work are admirable. Her enthusiasm in the form of overly optimistic project schedules is unforgettable.

I am greatly indebted to Dr. David B. Allison and Prof. Markku Savolainen for carefully reviewing the manuscript of my thesis and for their valuable comments and criticism. M.Sc. Donald Smart is thanked for reviewing the language of my thesis.

I wish to thank all the patients that participated to this study and thus made it possible. Especially, the twin pairs of the Finnish Twin Cohort have played a crucial role in the accomplishment of this work.

I am grateful for my collaborators, which have offered their intellectual opinions and ideas to improve and enhance this work. Prof. Kimmo Kontula, Prof. Jaakko Kaprio, Prof. Markku Koskenvuo, Prof. Olli Jänne, Dos. Pertti Mustajoki, Dr. Kati Kainulainen and Dr. Laura Oksanen are thanked for many fruitful meetings and discussions. Especially, I want to thank Prof. Jaakko Kaprio for always providing his help and a quick answer to my questions. I'm also grateful for his very constructive criticism and comments for the manuscript of my thesis. Dr. Laura Oksanen has shared not only concerns of a young scientist but also nice congress trips with me. I owe many thanks to Dr. Joseph D. Terwilliger for offering his help in statistical problems and for giving valuable comments for my studies. I wish to thank Drs. Veikko Koivisto, Mark Heiman, Aila Rissanen, Jorma Salmi, Markus Perola, Maija Wessman, Jenni Leppävuori, Päivi Pajukanta, Aarno Palotie and Kenneth Lange for collaboration.

Drs. Anu Jalanko and Ismo Ulmanen, the heads of the Department, have been "fair players" and very helpful, and are readily acknowledged for it. I sincerely wish to thank Tero Hiekkalinna, Teemu Perheentupa and Aki Suomalainen for patiently helping me in various issues related to computing and database management, and dedicating themselves to resolve my problems when my tolerance for computers was zero. Sari Kivikko and Sari Enckell are thanked for their skillful help in many practical matters. For expert technical assistance or excellent advice and help related to laboratory work I warmly thank Elli Kempas, Soili Johansson, Arja Terola, Pekka Ellonen, Jaana Hartiala, Maikki Parkkonen, Anne Jokiaho and Mari Sipilä.

I want to express my gratitude for my lab mates in LMGO for making the time spent there quite tolerable and sometimes even great fun. Jyrki Kaukonen has contributed remarkably to my getting-along in lab with his peculiar sense of humor and extraordinary jokes. Original amusement creators also include Lasse Lönnqvist, Markus Perola and Tomi Pastinen. Jenni Leppävuori and Iiris Hovatta have offered a great company during a congress trip and many cheerful moments in lab. There are several persons that have been building together the good old LMGO atmosphere, shared the problems of junior scientist or in a friendly way helped me during these long years. I'll

warmly remember all of you: Johanna, Satu, Hannele, Tiina, Paulina, Päivi M-B, Tarska, Markus, Lasse, Jyrki, Tomi, Petra P, Iiris, Jenni, Henna, Jesper, Teppo, Pekka and Mervi.

My life outside the lab would have been much more boring without my dearest friends, their existence have been vitally important. Their willingness to remind me of things not-related-to-lab has been priceless. I owe my sincerest thanks to Maarit for our long friendship and for her amazing ability to listen and give support. Kati is warmly thanked for tolerating my impossible timetables and still offering me delicious tiramisu. I have shared many memorable moments with Minna and I am pleased to be the godmother of his son. Eeva has "rescued" me countless times in Medical School. Papu, Ulla, Suski, Jonna, Mervi, Tuikku and Hansku, you have kept me up with drinking tea regularly for already ten years! Much of the energy required to accomplish this work has come to me from skiing, and that is why I'm grateful for all my friends that have shared this passion with me. I also want to thank our adventure racing team for numerous fun and not-so-fun training sessions and competitions together – they have offered a great way to grow one's patience!

Love is an incredible source of energy. I want to express my deepest thanks to Jussi for his true love and the incessant encouragement he has given to me. Without his incitement, the final phase of this work would have been much heavier to go through.

Finally, I am most indebted to my dear family. I do owe my sincerest thanks to my parents Helena and Kaj, who have been encouraging me continuously through these years. My brothers Kalle and Ville have been valuable listeners and supporters during difficult times and tolerated my temper surprisingly well. The endless love, care and support from my family have been in greatest importance to me. I wish I could thank you enough.

This study was financially supported by the Finnish Heart Foundation, the University of Helsinki, the Finnish Medical Foundation, the Open Care Research Foundation, the Paulo Foundation, the Aarne Koskelo Foundation, the Maud Kuistila Foundation and the Farmos and Orion Research Foundation, which are gratefully acknowledged.

Helsinki, March 2001

Miina Öhman

REFERENCES

Abbott A (2000) Manhattan versus Reykjavik. Nature 406:340-2

Ahima RS, Flier JS (2000) Leptin. Annual Review of Physiology 62:413-37

Allison DB, Faith MS, Heo M, Townsend-Butterworth D, Williamson DF (1999a) Meta-analysis of the effect of excluding early deaths on the estimated relationship between body mass index and mortality. Obesity Research 7:342-54

Allison DB, Faith MS, Nathan JS (1996) Risch's lambda values for human obesity. International Journal of Obesity & Related Metabolic Disorders 20:990-9

Allison DB, Heo M (1998b) Meta-analysis of linkage data under worst-case conditions: a demonstration using the human OB region. Genetics 148:859-65

Allison DB, Heo M, Schork NJ, Wong SL, Elston RC (1998a) Extreme selection strategies in gene mapping studies of oligogenic quantitative traits do not always increase power. Human Heredity 48:97-107

Allison DB, Heshka S, Neale MC, Lykken DT, Heymsfield SB (1994) A genetic analysis of relative weight among 4,020 twin pairs, with an emphasis on sex effects. Health Psychology 13:362-5

Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, Hayakawa K (1996b) The heritability of body mass index among an international sample of monozygotic twins reared apart. International Journal of Obesity & Related Metabolic Disorders 20:501-506

Allison DB, Neale MC, Zannolli R, Schork NJ, Amos CI, Blangero J (1999c) Testing the robustness of the likelihood-ratio test in a variance-component quantitative-trait loci-mapping procedure. American Journal of Human Genetics 65:531-44

Allison DB, Zannolli R, Faith MS, Heo M, Pietrobelli A, VanItallie TB, Pi-Sunyer FX, Heymsfield SB (1999b) Weight loss increases and fat loss decreases all-cause mortality rate: results from two independent cohort studies. International Journal of Obesity & Related Metabolic Disorders 23:603-11

Almasy L, Blangero J (1998) Multipoint quantitative-trait linkage analysis in general pedigrees. American Journal of Human Genetics 62:1198-211

Amos CI, Elston RC (1989) Robust methods for the detection of genetic linkage for quantitative data from pedigrees [published erratum appears in Genet Epidemiol 1989;6(6):727]. Genetic Epidemiology 6:349-60

Anderson JL, Khan M, David WS, Mahdavi Z, Nuttall FQ, Krech E, West SG, Vance JM, Pericak-Vance MA, Nance MA (1999) Confirmation of linkage of hereditary partial lipodystrophy to chromosome 1q21-22. American Journal of Medical Genetics 82:161-5

Andersson L, Haley CS, Ellegren H, Knott SA, Johansson M, Andersson K, Andersson-Eklund L, Edfors-Lilja I, Fredholm M, Hansson I, et al. (1994) Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science 263:1771-4

Arnason E, Sigurgislason H, Benedikz E (2000) Genetic homogeneity of Icelanders: fact or fiction? Nature Genetics 25:373-4

Axelrod J, Reisine TD (1984) Stress hormones: their interaction and regulation. Science 224:452-9

Bamshad M, Lin RC, Law DJ, Watkins WC, Krakowiak PA, Moore ME, Franceschini P, Lala R, Holmes LB, Gebuhr TC, Bruneau BG, Schinzel A, Seidman JG, Seidman CE, Jorde LB (1997) Mutations in human TBX3 alter limb, apocrine and genital development in ulnar-mammary syndrome [published erratum appears in Nat Genet 1998 May;19(1):102]. Nature Genetics 16:311-5

Banauch D, Brummer W, Ebeling W, Metz H, Rindfrey H, Lang H, Leybold K, Rick W, Staudinger HJ (1975) A glucose dehydrogenase for the determination of glucose concentrations in body fluids (author's transl). Zeitschrift fur Klinische Chemie und Klinische Biochemie 13:101-7

Banerjee P, Kleyn PW, Knowles JA, Lewis CA, Ross BM, Parano E, Kovats SG, Lee JJ, Penchaszadeh

GK, Ott J, Jacobson SG, Gilliam TC (1998) TULP1 mutation in two extended Dominican kindreds with autosomal recessive retinitis pigmentosa. Nature Genetics 18:177-9

Baskin DG, Schwartz MW, Seeley RJ, Woods SC, Porte D, Jr., Breininger JF, Jonak Z, Schaefer J, Krouse M, Burghardt C, Campfield LA, Burn P, Kochan JP (1999) Leptin receptor long-form splice-variant protein expression in neuron cell bodies of the brain and co-localization with neuropeptide Y mRNA in the arcuate nucleus. Journal of Histochemistry & Cytochemistry 47:353-62

Baxter G, Kennett G, Blaney F, Blackburn T (1995) 5-HT2 receptor subtypes: a family re-united? Trends in Pharmacological Sciences 16:105-10

Behr M, Ramsden DB, Loos U (1997) Deoxyribonucleic acid binding and transcriptional silencing by a truncated c-erbA beta 1 thyroid hormone receptor identified in a severely retarded patient with resistance to thyroid hormone. Journal of Clinical Endocrinology & Metabolism 82:1081-7

Bertagna X (1994) Proopiomelanocortin-derived peptides. Endocrinology & Metabolism Clinics of North America 23:467-85

Blangero J, Almasy L (1997) Multipoint oligogenic linkage analysis of quantitative traits. Genetic Epidemiology 14:959-64

Blangero J, Williams JT, Almasy L (2000) Quantitative trait locus mapping using human pedigrees. Human Biology 72:35-62

Blundell JE, Lawton CL, Halford JC (1995) Serotonin, eating behavior, and fat intake. Obesity Research 3:471S-476S

Boggon TJ, Shan WS, Santagata S, Myers SC, Shapiro L (1999) Implication of tubby proteins as transcription factors by structure-based functional analysis. Science 286:2119-25

Borecki IB, Rice T, Bouchard C, Rao DC (1991) Commingling analysis of generalized body mass and composition measures: the Quebec Family Study. International Journal of Obesity 15:763-73

Bouchard C (1995) The genetics of obesity: from genetic epidemiology to molecular markers. Molecular Medicine Today 1:45-50

Bouchard C, Perusse L, Leblanc C, Tremblay A, Theriault G (1988) Inheritance of the amount and distribution of human body fat. International Journal of Obesity 12:205-15

Bray GA (1995) Laurence, Moon, Bardet, and Biedl: reflections on a syndrome. Obesity Research 3:383-6

Bray GA, Popkin BM (1998) Dietary fat intake does affect obesity. American Journal of Clinical Nutrition 68:1157-73

Brzustowicz LM, Farrell S, Khan MB, Weksberg R (1999) Mapping of a new SGBS locus to chromosome Xp22 in a family with a severe form of Simpson-Golabi-Behmel syndrome. American Journal of Human Genetics 65:779-83

Bultman SJ, Michaud EJ, Woychik RP (1992) Molecular characterization of the mouse agouti locus. Cell 71:1195-1204

Burnet PW, Smith KA, Cowen PJ, Fairburn CG, Harrison PJ (1999) Allelic variation of the 5-HT2C receptor (HTR2C) in bulimia nervosa and binge eating disorder. Psychiatric Genetics 9:101-4

Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW, Jr. (1999) Body-mass index and mortality in a prospective cohort of U.S. adults. New England Journal of Medicine 341:1097-105

Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546-9

Cardon LR, Carmelli D, Fabsitz RR, Reed T (1994) Genetic and environmental correlations between obesity and body fat distribution in adult male twins. Human Biology 66:465-79

Cardon LR, Fulker DW (1994) The power of interval mapping of quantitative trait loci, using selected sib pairs. American Journal of Human Genetics 55:825-33

Cederlof R, Rantasalo I, Floderus-Myrhed B, Hammar N, Kaprio J, Koskenvuo M, Langinvainio H, Sarna S (1982) A cross-national epidemiological resource: the Swedish and Finnish cohort studies of

like-sexed twins. International Journal of Epidemiology 11:387-90

Chagnon YC, Chen WJ, Perusse L, Chagnon M, Nadeau A, Wilkison WO, Bouchard C (1997) Linkage and Association Studies Between the Melanocortin Receptors 4 and 5 Genes and Obesity-Related Phenotypes in the Quebec Family Study. Molecular Medicine 3:663-673

Chagnon YC, Perusse L, Lamothe M, Chagnon M, Nadeau A, Dionne FT, Gagnon J, Chung WK, Leibel RL, Bouchard C (1997) Suggestive linkages between markers on human 1p32-p22 and body fat and insulin levels in the Quebec Family Study. Obesity Research 5:115-21

Chagnon YC, Perusse L, Weisnagel SJ, Rankinen T, Bouchard C (2000) The human obesity gene map: the 1999 update. Obesity Research 8:89-117

Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC (1994) Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care 17:961-9

Chehab FF (1997) The reproductive side of leptin. Nature Medicine 3:952-3

Chehab FF, Lim ME, Lu R (1996) Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nature Genetics 12:318-20

Chen AS, Marsh DJ, Trumbauer ME, Frazier EG, Guan XM, Yu H, Rosenblum CI, Vongs A, Feng Y, Cao LH, Metzger JM, Strack AM, Camacho RE, Mellin TN, Nunes CN, Min W, Fisher J, Gopal-Truter S, MacIntyre DE, Chen HY, Van der Ploeg LHT (2000) Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. Nature Genetics 26:97-102

Cheung CC, Clifton DK, Steiner RA (1997) Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. Endocrinology 138:4489-92

Chotai J (1984) On the lod score method in linkage analysis. Annals of Human Genetics 48:359-78

Chung W, Goldberg-Berman J, Power-Kehoe L, Leibel RL (1996) Molecular mapping of the tubby (tub) mutation on mouse chromosome 7. Genomics 32:210-217

Clement K, Garner C, Hager J, Philippi A, LeDuc C, Carey A, Harris TJ, Jury C, Cardon LR, Basdevant A, Demenais F, Guy-Grand B, North M, Froguel P (1996) Indication for linkage of the human OB gene region with extreme obesity. Diabetes 45:687-690

Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guygrand B (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392:398-401

Colditz GA (1999) Economic costs of obesity and inactivity. Medicine & Science in Sports & Exercise 31:S663-7

Colditz GA, Willett WC, Rotnitzky A, Manson JE (1995) Weight gain as a risk factor for clinical diabetes mellitus in women. Annals of Internal Medicine 122:481-6

Colditz GA, Willett WC, Stampfer MJ, Manson JE, Hennekens CH, Arky RA, Speizer FE (1990) Weight as a risk factor for clinical diabetes in women. American Journal of Epidemiology 132:501-13

Coleman DL (1973) Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 9:294-8

Coleman DL, Eicher EM (1990) Fat (fat) and tubby (tub): two autosomal recessive mutations causing obesity syndromes in the mouse. Journal of Heredity 81:424-7

Comuzzie AG, Almasy L, Cole SA, Boss O, Giacobino JP, Muzzin P, Stern MP, MacCluer JW, Blangero J, Hixson JE (2000) Linkage exclusion analysis of the chromosome 11 region containing UCP2 and UCP3 with obesity-related phenotypes in Mexican Americans. International Journal of Obesity 24:1065-1068

Comuzzie AG, Hixson JE, Almasy L, Mitchell BD, Mahaney MC, Dyer TD, Stern MP, MacCluer JW, Blangero J (1997) A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. Nature Genetics 15:273-276

Cone RD (2000) Haploinsufficiency of the melanocortin-4 receptor: part of a thrifty genotype? Journal of

Clinical Investigation 106:185-7

Cone RD, Lu D, Koppula S, Vage DI, Klungland H, Boston B, Chen W, Orth DN, Pouton C, Kesterson RA (1996) The melanocortin receptors: agonists, antagonists, and the hormonal control of pigmentation. Recent Progress in Hormone Research 51:287-317; discussion 318

Considine RV, Considine EL, Williams CJ, Nyce MR, Magosin SA, Bauer TL, Rosato EL, Colberg J, Caro JF (1995) Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. Journal of Clinical Investigation 95:2986-8

Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, et al. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. New England Journal of Medicine 334:292-5

Consortium IHGS (2001) Initial sequencing and analysis of the human genome. Nature 409:860-921

Cox DR, Burmeister M, Price ER, Kim S, Myers RM (1990) Radiation hybrid mapping: a somatic cell genetic method for constructing high-resolution maps of mammalian chromosomes. Science 250:245-50

Crow JF, Kimura M (1970) An Introduction to Population Genetics Theory. Harper & Row, New York, NY

Cummings DE, Schwartz MW (2000) Melanocortins and body weight: a tale of two receptors. Nature Genetics 26:8-9

Curzon G, Gibson EL, Oluyomi AO (1997) Appetite suppression by commonly used drugs depends on 5-HT receptors but not on 5-HT availability. Trends in Pharmacological Sciences 18:21-5

Davies PS, Gregory J, White A (1995) Physical activity and body fatness in pre-school children. International Journal of Obesity & Related Metabolic Disorders 19:6-10

de la Chapelle A, Wright FA (1998) Linkage disequilibrium mapping in isolated populations: the example of Finland revisited. Proceedings of the National Academy of Sciences of the United States of America 95:12416-23

den Tonkelaar I, Seidell JC, Collette HJ (1995) Body fat distribution in relation to breast cancer in women participating in the DOM-project. Breast Cancer Research & Treatment 34:55-61

Desbuquois B, Aurbach GD (1971) Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. Journal of Clinical Endocrinology & Metabolism 33:732-8

Dinulescu DM, Cone RD (2000) Agouti and agouti-related protein: analogies and contrasts. Journal of Biological Chemistry 275:6695-8

Dinulescu DM, Fan W, Boston BA, McCall K, Lamoreux ML, Moore KJ, Montagno J, Cone RD (1998) Mahogany (mg) stimulates feeding and increases basal metabolic rate independent of its suppression of agouti. Proceedings of the National Academy of Sciences of the United States of America 95:12707-12

Dionne I, Despres JP, Bouchard C, Tremblay A (1999) Gender difference in the effect of body composition on energy metabolism. International Journal of Obesity & Related Metabolic Disorders 23:312-9

Dourish CT (1995) Multiple serotonin receptors: opportunities for new treatments for obesity? Obesity Research 3:449S-462S

Duhl DM, Stevens ME, Vrieling H, Saxon PJ, Miller MW, Epstein CJ, Barsh GS (1994) Pleiotropic effects of the mouse lethal yellow (Ay) mutation explained by deletion of a maternally expressed gene and the simultaneous production of agouti fusion RNAs. Development 120:1695-708

Eaves IA, Merriman TR, Barber RA, Nutland S, Tuomilehto-Wolf E, Tuomilehto J, Cucca F, Todd JA (2000) The genetically isolated populations of Finland and Sardinia may not be a panacea for linkage disequilibrium mapping of common disease genes. Nature Genetics 25:320-3

Eckel RH (1997) Obesity and heart disease: a statement for healthcare professionals from the Nutrition

Committee, American Heart Association. Circulation 96:3248-50

Elmquist JK, Elias CF, Saper CB (1999) From lesions to leptin: hypothalamic control of food intake and body weight. Neuron 22:221-32

Esparza J, Fox C, Harper IT, Bennett PH, Schulz LO, Valencia ME, Ravussin E (2000) Daily energy expenditure in Mexican and USA Pima indians: low physical activity as a possible cause of obesity. International Journal of Obesity & Related Metabolic Disorders 24:55-9

Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD (1997) Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385:165-8

Faraway JJ (1993) Improved sib-pair linkage test for disease susceptibility loci [published erratum appears in Genet Epidemiol 1994;11(1):99]. Genetic Epidemiology 10:225-33

Farooqi IS, Yeo GSH, Keogh JM, Aminian S, Jebb SA, Butler G, Cheetham T, O'Rahilly S (2000) Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. Journal of Clinical Investigation 106:271-279

Ferrari W (1958) Behavioral changes in animals after intracisternal injection with adrenocorticotrophic hormone and melanocyte-stimulating hormone. Nature 181:925-926

Finley PR, Schifman RB, Williams RJ, Lichti DA (1978) Cholesterol in high-density lipoprotein: use of Mg2+/dextran sulfate in its enzymic measurement. Clinical Chemistry 24:931-3

Finn PD, Cunningham MJ, Pau KY, Spies HG, Clifton DK, Steiner RA (1998) The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrinology 139:4652-62

Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL (1998) Overweight and obesity in the United States: prevalence and trends, 1960-1994. International Journal of Obesity & Related Metabolic Disorders 22:39-47

Flier JS, Maratos-Flier E (1998) Obesity and the hypothalamus: novel peptides for new pathways. Cell 92:437-40

Forsen T, Eriksson J, Qiao Q, Tervahauta M, Nissinen A, Tuomilehto J (2000) Short stature and coronary heart disease: a 35-year follow-up of the Finnish cohorts of The Seven Countries Study. Journal of Internal Medicine 248:326-332

Frankel WN, Schork NJ (1996) Who's afraid of epistasis? Nature Genetics 14:371-3

Fulker DW, Cherny SS (1996) An improved multipoint sib-pair analysis of quantitative traits. Behavior Genetics 26:527-32

Gallagher D, Visser M, Sepulveda D, Pierson RN, Harris T, Heymsfield SB (1996) How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? American Journal of Epidemiology 143:228-39

Gantz I, Miwa H, Konda Y, Shimoto Y, Tashiro T, Watson SJ, DelValle J, Yamada T (1993) Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. Journal of Biological Chemistry 268:15174-15179

Garg A, Wilson R, Barnes R, Arioglu E, Zaidi Z, Gurakan F, Kocak N, O'Rahilly S, Taylor SI, Patel SB, Bowcock AM (1999) A gene for congenital generalized lipodystrophy maps to human chromosome 9q34. Journal of Clinical Endocrinology & Metabolism 84:3390-4

Gecz J, Baker E, Donnelly A, Ming JE, McDonald-McGinn DM, Spinner NB, Zackai EH, Sutherland GR, Mulley JC (1999) Fibroblast growth factor homologous factor 2 (FHF2): gene structure, expression and mapping to the Borjeson-Forssman-Lehmann syndrome region in Xq26 delineated by a duplication breakpoint in a BFLS-like patient. Human Genetics 104:56-63

Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC (1996) Defective STAT signaling by the leptin receptor in diabetic mice. Proceedings of the National Academy of Sciences of the United States of America 93:6231-5

Ghosh S, Schork NJ (1996) Genetic analysis of NIDDM. The study of quantitative traits. Diabetes 45:1-14

Gill RW, Sanseau P (2000) Rapid in silico cloning of genes using expressed sequence tags (ESTs).

Biotechnology Annual Review 5:25-44

Gillessen-Kaesbach G, Demuth S, Thiele H, Theile U, Lich C, Horsthemke B (1999) A previously unrecognised phenotype characterised by obesity, muscular hypotonia, and ability to speak in patients with Angelman syndrome caused by an imprinting defect [see comments]. European Journal of Human Genetics 7:638-44

Goldbourt U, Medalie JH (1974) Weight-height indices. Choice of the most suitable index and its association with selected variables among 10,000 adult males of heterogeneous origin. British Journal of Preventive & Social Medicine 28:116-26

Goldgar DE (1990) Multipoint analysis of human quantitative genetic variation. American Journal of Human Genetics 47:957-67

Gorman SW, Haider NB, Grieshammer U, Swiderski RE, Kim E, Welch JW, Searby C, Leng S, Carmi R, Sheffield VC, Duhl DM (1999) The cloning and developmental expression of unconventional myosin IXA (MYO9A) a gene in the Bardet-Biedl syndrome (BBS4) region at chromosome 15q22-q23. Genomics 59:150-60

Gotoda T, Manning BS, Goldstone AP, Imrie H, Evans AL, Strosberg AD, McKeigue PM, Scott J, Aitman TJ (1997) Leptin receptor gene variation and obesity: lack of association in a British male population. Human Molecular Genetics 6:869-876

Gotoda T, Scott J, Aitman TJ (1997) Molecular screening of the human melanocortin-4 receptor gene: identification of a missense variant showing no association with obesity, plasma glucose, or insulin. Diabetologia 40:976-9

Graham M, Shutter JR, Sarmiento U, Sarosi I, Stark KL (1997) Overexpression of Agrt leads to obesity in transgenic mice. Nature Genetics 17:273-4

Gray DS, Fujioka K (1991) Use of relative weight and Body Mass Index for the determination of adiposity. Journal of Clinical Epidemiology 44:545-50

Grol ME, Eimers JM, Alberts JF, Bouter LM, Gerstenbluth I, Halabi Y, van Sonderen E, van den Heuvel WJ (1997) Alarmingly high prevalence of obesity in Curacao: data from an interview survey stratified for socioeconomic status. International Journal of Obesity & Related Metabolic Disorders 21:1002-9

Groot PC, Moen CJ, Dietrich W, Stoye JP, Lander ES, Demant P (1992) The recombinant congenic strains for analysis of multigenic traits: genetic composition. FASEB Journal 6:2826-35

Gu C, Province M, Rao DC (1999b) Meta-analysis of genetic linkage to quantitative trait loci with study-specific covariates: a mixed-effects model. Genetic Epidemiology 17:S599-604

Gu W, Tu Z, Kleyn PW, Kissebah A, Duprat L, Lee J, Chin W, Maruti S, Deng N, Fisher SL, Franco LS, Burn P, Yagaloff KA, Nathan J, Heymsfield S, Albu J, Pi-Sunyer FX, Allison DB (1999) Identification and functional analysis of novel human melanocortin-4 receptor variants. Diabetes 48:635-9

Göring HHH, Terwilliger JD (2000c) Linkage analysis in the presence of errors IV: Joint pseudomarker analysis of linkage and/or linkage disequilibrium on a mixture of pedigrees and singletons when the mode of inheritance cannot be accurately specified. American Journal of Human Genetics 66:1310-1327

Hager J, Dina C, Francke S, Dubois S, Houari M, Vatin V, Vaillant E, Lorentz N, Basdevant A, Clement K, Guy-Grand B, Froguel P (1998) A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. Nature Genetics 20:304-8

Hagstrom SA, North MA, Nishina PL, Berson EL, Dryja TP (1998) Recessive mutations in the gene encoding the tubby-like protein TULP1 in patients with retinitis pigmentosa. Nature Genetics 18:174-6

Hakala P, Rissanen A, Koskenvuo M, Kaprio J, Ronnemaa T (1999) Environmental factors in the development of obesity in identical twins. International Journal of Obesity & Related Metabolic Disorders 23:746-53

Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-6

Hall C, Manser E, Spurr NK, Lim L (1993) Assignment of the human carboxypeptidase E (CPE) gene to chromosome 4. Genomics 15:461-3

Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennett PH, Knowler WC (1998) An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. American Journal of Human Genetics 63:1130-8

Harris JR, Tambs K, Magnus P (1995) Sex-specific effects for body mass index in the new Norwegian twin panel. Genetic Epidemiology 12:251-65

Harvey J, McKenna F, Herson PS, Spanswick D, Ashford ML (1997) Leptin activates ATP-sensitive potassium channels in the rat insulin-secreting cell line, CRI-G1. Journal of Physiology 504:527-35

Haseman JK, Elston RC (1972) The investigation of linkage between a quantitative trait and a marker locus. Behavior Genetics 2:3-19

Hasstedt SJ, Hoffman M, Leppert MF, Elbein SC (1997) Recessive inheritance of obesity in familial non-insulin-dependent diabetes mellitus, and lack of linkage to nine candidate genes. American Journal of Human Genetics 61:668-77

Hausberger FX (1959) Parabiosis and transplantation experiments in heriditary obese mice. Anat. Rec. 130:313

Hedeland H, Berntorp K, Arheden K, Kristoffersson U (1992) Pseudohypoparathyroidism type I and Albright's hereditary osteodystrophy with a proximal 15q chromosomal deletion in mother and daughter. Clinical Genetics 42:129-34

Heisler LK, Chu HM, Tecott LH (1998) Epilepsy and obesity in serotonin 5-HT2C receptor mutant mice. Annals of the New York Academy of Sciences 861:74-8

Heitmann BL, Erikson H, Ellsinger BM, Mikkelsen KL, Larsson B (2000) Mortality associated with body fat, fat-free mass and body mass index among 60-year-old swedish men-a 22-year follow-up. The study of men born in 1913. International Journal of Obesity & Related Metabolic Disorders 24:33-7

Heitmann BL, Kaprio J, Harris JR, Rissanen A, Korkeila M, Koskenvuo M (1997) Are genetic determinants of weight gain modified by leisure-time physical activity? A prospective study of Finnish twins. American Journal of Clinical Nutrition 66:672-8

Herskind AM, McGue M, Sorensen TI, Harvald B (1996) Sex and age specific assessment of genetic and environmental influences on body mass index in twins. International Journal of Obesity & Related Metabolic Disorders 20:106-13

Hetherington AW, Ranson SW (1940) Hypothalamic lesions and adiposity in the rat. Anat. Rec. 78:149-172

Hill JO, Peters JC (1998) Environmental contributions to the obesity epidemic. Science 280:1371-4

Hinney A, Schmidt A, Nottebom K, Heibult O, Becker I, Ziegler A, Gerber G, Sina M, Gorg T, Mayer H, Siegfried W, Fichter M, Remschmidt H, Hebebrand J (1999) Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. Journal of Clinical Endocrinology & Metabolism 84:1483-6

Hirschhorn JN, Lindgren CM, Daly MJ, Kirby A, Schaffner SF, Altshuler D, Burtt NP, Parker A, Rioux JD, Platko J, Gaudet D, Hudson TJ, Groop LC, Lander ES (2001 submitted) Genome-wide linkage analysis of stature in multiple populations reveals several regions with evidence of linkage to adult height.

Hodge AM, Dowse GK, Gareeboo H, Tuomilehto J, Alberti KG, Zimmet PZ (1996) Incidence, increasing

prevalence, and predictors of change in obesity and fat distribution over 5 years in the rapidly developing population of Mauritius. International Journal of Obesity & Related Metabolic Disorders 20:137-46

Holmans P, Craddock N (1997) Efficient strategies for genome scanning using maximum-likelihood affected-sib-pair analysis. American Journal of Human Genetics 60:657-66

Hubert HB, Feinleib M, McNamara PM, Castelli WP (1983) Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation 67:968-77

Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:131-141

Ingalls AM, Dickie MM, Snell GD (1950) Obesity, a new mutation in the house mouse. Journal of Heredity 41:317-318

Jackson RS, Creemers JWM, Ohagi S, Raffin-Sanson M-L, Sanders L, Montague CT, Hutton JC, O'Rahilly S (1997) Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nature Genetics 16:303-306

Jimerson DC, Wolfe BE, Metzger ED, Finkelstein DM, Cooper TB, Levine JM (1997) Decreased serotonin function in bulimia nervosa. Archives of General Psychiatry 54:529-34

Kahn HS, Williamson DF (1990) The contributions of income, education and changing marital status to weight change among US men. International Journal of Obesity 14:1057-68

Kainulainen K, Perola M, Terwilliger J, Kaprio J, Koskenvuo M, Syvänen AC, Vartiainen E, Peltonen L, Kontula K (1999) Evidence for involvement of the type 1 angiotensin II receptor locus in essential hypertension. Hypertension 33:844-9

Kapeller R, Moriarty A, Strauss A, Stubdal H, Theriault K, Siebert E, Chickering T, Morgenstern JP, Tartaglia LA, Lillie J (1999) Tyrosine phosphorylation of tub and its association with Src homology 2 domain-containing proteins implicate tub in intracellular signaling by insulin. Journal of Biological Chemistry 274:24980-6

Kaprio J (1994) Lessons from twin studies in Finland. Annals of Medicine 26:135-9

Kaprio J, Sarna S, Koskenvuo M, Rantasalo I (1978) The Finnish Twin Registry: formation and compilation, questionnaire study, zygosity determination procedures, and research program. Progress in Clinical & Biological Research 24:179-84

Katsanis N, Beales PL, Woods MO, Lewis RA, Green JS, Parfrey PS, Ansley SJ, Davidson WS, Lupski JR (2000) Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet-Biedl syndrome. Nature Genetics 26:67-70

Kennedy GC (1953) The role of depot fat in the control of food intake in the rat. Proc. R. Soc. London Ser. B. 140:578-596

Khosla T, Lowe CR (1967) Indices of obesity derived from body weight and height. British Journal of Preventive & Social Medicine 21:122-8

Kim H, Kadowaki H, Sakura H, Odawara M, Momomura K, Takahashi Y, Miyazaki Y, Ohtani T, Akanuma Y, Yazaki Y, et al. (1992) Detection of mutations in the insulin receptor gene in patients with insulin resistance by analysis of single-stranded conformational polymorphisms. Diabetologia 35:261-6

Kim KS, Larsen N, Short T, Plastow G, Rothschild MF (2000) A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. Mammalian Genome 11:131-135

Kissebah AH, Krakower GR (1994) Regional adiposity and morbidity. Physiological Reviews 74:761-811

Kittles RA, Perola M, Peltonen L, Bergen AW, Aragon RA, Virkkunen M, Linnoila M, Goldman D, Long JC (1998) Dual origins of Finns revealed by Y chromosome haplotype variation. American

Journal of Human Genetics 62:1171-9

Kleyn PW, Fan W, Kovats SG, Lee JJ, Pulido JC, Wu Y, Berkemeier LR, Misumi DJ, Holmgren L, Charlat O, Woolf EA, Tayber O, Brody T, Shu P, Hawkins F, Kennedy B, Baldini L, Ebeling C, Alperin GD, Deeds J, Lakey ND, Culpepper J, Chen H, Glucksmann-Kuis MA, Carlson GA, Duyk GM, Moore KJ (1996) Identification and Characterization of the Mouse Obesity Gene tubby: A Member of a Novel Gene Family. Cell 85:281-290

Knapp M, Seuchter SA, Baur MP (1993) The haplotype-relative-risk (HRR) method for analysis of association in nuclear families. American Journal of Human Genetics 52:1085-93

Kopelman PG (2000) Obesity as a medical problem. Nature 404:635-43

Kopelman PG (1992) Sleep apnoea and hypoventilation in obesity. International Journal of Obesity & Related Metabolic Disorders 16:S37-42

Korkeila M, Kaprio J, Rissanen A, Koskenvuo M (1991) Effects of gender and age on the heritability of bofy mass index. International Journal of Obesity & Related Metabolic Disorders 15:647-654

Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S (1998) Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 393:72-6

Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A (1998) Severe Early-Onset Obesity, Adrenal Insufficiency and Red Hair Pigmentation Caused By Pomc Mutations in Humans. Nature Genetics 19:155-157

Kruglyak L (1999) Prospects for whole-genome linkage disequilibrium mapping of common disease genes. Nature Genetics 22:139-44

Kruglyak L, Lander ES (1995) Complete multipoint sib-pair analysis of qualitative and quantitative traits. American Journal of Human Genetics 57:439-54

Kruglyak L, Lander ES (1995) High-resolution genetic mapping of complex traits [published erratum appears in Am J Hum Genet 1996 Apr;58(4):902]. American Journal of Human Genetics 56:1212-23

Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL (1994) Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys, 1960 to 1991. Jama 272:205-11

Kuokkanen S, Sundvall M, Terwilliger JD, Tienari PJ, Wikström J, Holmdahl R, Pettersson U, Peltonen L (1996) A putative vulnerability locus to multiple sclerosis maps to 5p14-p12 in a region syntenic to the murine locus Eae2. Nature Genetics 13:477-480

Kusin JA, Kardjati S, Houtkooper JM, Renqvist UH (1992) Energy supplementation during pregnancy and postnatal growth. Lancet 340:623-6

Kwitek-Black AE, Carmi R, Duyk GM, Buetow KH, Elbedour K, Parvari R, Yandava CN, Stone EM, Sheffield VC (1993) Linkage of Bardet-Biedl syndrome to chromosome 16q and evidence for non-allelic genetic heterogeneity. Nature Genetics 5:392-6

Kwon HY, Bultman SJ, Loffler C, Chen WJ, Furdon PJ, Powell JG, Usala AL, Wilkison W, Hansmann I, Woychik RP (1994) Molecular structure and chromosomal mapping of the human homolog of the agouti gene. Proceedings of the National Academy of Sciences of the United States of America 91:9760-4

Lahti-Koski M, Vartiainen E, Mannisto S, Pietinen P (2000) Age, education and occupation as determinants of trends in body mass index in Finland from 1982 to 1997. International Journal of Obesity 24:1669-1676

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genetics 11:241-7

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps [published erratum appears in Genetics 1994 Feb;136(2):705]. Genetics 121:185-99

Lander ES, Schork NJ (1994) Genetic dissection of complex traits [published erratum appears in Science 1994 Oct 21;266(5184):353]. Science 265:2037-48

Lange K, Weeks D, Boehnke M (1988) Programs for Pedigree Analysis: MENDEL, FISHER, and dGENE. Genetic Epidemiology 5:471-2

Lappalainen J, Zhang L, Dean M, Oz M, Ozaki N, Yu DH, Virkkunen M, Weight F, Linnoila M, Goldman D (1995) Identification, expression, and pharmacology of a Cys23-Ser23 substitution in the human 5-HT2c receptor gene (HTR2C). Genomics 27:274-9

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proceedings of the National Academy of Sciences of the United States of America 81:3443-6

Lauer MS, Anderson KM, Kannel WB, Levy D (1991) The impact of obesity on left ventricular mass and geometry. The Framingham Heart Study. Jama 266:231-6

Laurier D, Guiguet M, Chau NP, Wells JA, Valleron AJ (1992) Prevalence of obesity: a comparative survey in France, the United Kingdom and the United States. International Journal of Obesity & Related Metabolic Disorders 16:565-72

Lee G-H, Proenca R, Montez JM, Carrol KM, Darvishzadeh JG, Lee JI, Friedman JM (1996) Abnormal splicing of the leptin receptor in diabetic mice. Nature 379:632-635

Lee JH, Reed DR, Li WD, Xu W, Joo EJ, Kilker RL, Nanthakumar E, North M, Sakul H, Bell C, Price RA (1999) Genome scan for human obesity and linkage to markers in 20q13. American Journal of Human Genetics 64:196-209

Lee JH, Reed DR, Price RA (1997) Familial risk ratios for extreme obesity: implications for mapping human obesity genes. International Journal of Obesity & Related Metabolic Disorders 21:935-40

Legradi G, Emerson CH, Ahima RS, Flier JS, Lechan RM (1997) Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. Endocrinology 138:2569-76

Leibel RL (1997) And finally, genes for human obesity. Nature Genetics 16:218-20

Leibowitz SF, Alexander JT (1998) Hypothalamic serotonin in control of eating behavior, meal size, and body weight. Biological Psychiatry 44:851-64

Lembertas AV, Perusse L, Chagnon YC, Fisler JS, Warden CH, Purcell-Huynh DA, Dionne FT, Gagnon J, Nadeau A, Lusis AJ, Bouchard C (1997) Identification of an obesity quantitative trait locus on mouse chromosome 2 and evidence of linkage to body fat and insulin on the human homologous region 20q. Journal of Clinical Investigation 100:1240-7

Lentes KU, Hinney A, Ziegler A, Rosenkranz K, Wurmser H, Barth N, Jacob K, Coners H, Mayer H, Grzeschik KH, Schafer H, Remschmidt H, Pirke KM, Hebebrand J (1997) Evaluation of a Cys23Ser mutation within the human 5-HT2C receptor gene: no evidence for an association of the mutant allele with obesity or underweight in children, adolescents and young adults. Life Sciences 61:L9-16

Leppävuori J, Kujala U, Kinnunen J, Kaprio J, Nissilä M, Heliövaara M, Klinger N, Partanen J, Terwilliger JD, Peltonen L (1999) Genome scan for predisposing loci for distal interphalangeal joint osteoarthritis: Evidence for a locus on 2q. American Journal of Human Genetics. 65:1060-1067

Lernmark A, Ott J (1998) Sometimes it's hot, sometimes it's not. Nature Genetics 19:213-4

Levy E, Levy P, Le Pen C, Basdevant A (1995) The economic cost of obesity: the French situation. International Journal of Obesity & Related Metabolic Disorders 19:788-92

Li WD, Joo EJ, Furlong EB, Galvin M, Abel K, Bell CJ, Price RA (2000) Melanocortin 3 receptor (MC3R) gene variants in extremely obese women. International Journal of Obesity 24:206-210

Li Z, Rao DC (1996) Random effects model for meta-analysis of multiple quantitative sibpair linkage studies. Genetic Epidemiology 13:377-83

Lindroos AK, Lissner L, Mathiassen ME, Karlsson J, Sullivan M, Bengtsson C, Sjostrom L (1997) Dietary

intake in relation to restrained eating, disinhibition, and hunger in obese and nonobese Swedish women. Obesity Research 5:175-82

Livingstone B (2000) Epidemiology of childhood obesity in Europe. European Journal of Pediatrics 159:S14-S34

Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394:897-901

Losonczy KG, Harris TB, Cornoni-Huntley J, Simonsick EM, Wallace RB, Cook NR, Ostfeld AM, Blazer DG (1995) Does weight loss from middle age to old age explain the inverse weight mortality relation in old age? American Journal of Epidemiology 141:312-21

Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, Luther M, Chen W, Woychik RP, Wilkison WO, et al. (1994) Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. Nature 371:799-802

Macari F, Lautier C, Girardet A, Dadoun F, Darmon P, Dutour A, Renard E, Bouvagnet P, Claustres M, Oliver C, Grigorescu F (1998) Refinement of genetic localization of the Alstrom syndrome on chromosome 2p12-13 by linkage analysis in a North African family. Human Genetics 103:658-61

Maes HH, Neale MC, Eaves LJ (1997) Genetic and environmental factors in relative body weight and human adiposity. Behavior Genetics 27:325-51

Maffei M, Stoffel M, Barone M, Moon B, Dammerman M, Ravussin E, Bogardus C, Ludwig DS, Flier JS, Talley M, et al. (1996) Absence of mutations in the human OB gene in obese/diabetic subjects. Diabetes 45:679-82

Maffeis C (2000) Aetiology of overweight and obesity in children and adolescents. European Journal of Pediatrics 159:S35-S44

Magenis RE, Smith L, Nadeau JH, Johnson KR, Mountjoy KG, Cone RD (1994) Mapping of the ACTH, MSH, and neural (MC3 and MC4) melanocortin receptors in the mouse and human. Mammalian Genome 5:503-8

Malhotra AK, Goldman D, Ozaki N, Rooney W, Clifton A, Buchanan RW, Breier A, Pickar D (1996) Clozapine response and the 5HT2C Cys23Ser polymorphism. Neuroreport 7:2100-2

Manson JE, Colditz GA, Stampfer MJ, Willett WC, Rosner B, Monson RR, Speizer FE, Hennekens CH (1990) A prospective study of obesity and risk of coronary heart disease in women. New England Journal of Medicine 322:882-9

Manson JE, Willett WC, Stampfer MJ, Colditz GA, Hunter DJ, Hankinson SE, Hennekens CH, Speizer FE (1995) Body weight and mortality among women. New England Journal of Medicine 333:677-85

Martin N, Boomsma D, Machin G (1997) A twin-pronged attack on complex traits. Nature Genetics 17:387-92

Matthijs G, Schollen E, Pardon E, Veiga-Da-Cunha M, Jaeken J, Cassiman JJ, Van Schaftingen E (1997) Mutations in PMM2, a phosphomannomutase gene on chromosome 16p13, in carbohydrate-deficient glycoprotein type I syndrome (Jaeken syndrome) [published erratum appears in Nat Genet 1997 Jul;16(3):316]. Nature Genetics 16:88-92

McCrory MA, Fuss PJ, Saltzman E, Roberts SB (2000) Dietary determinants of energy intake and weight regulation in healthy adults. Journal of Nutrition 130:276S-279S

Michaud EJ, Bultman SJ, Klebig ML, van Vugt MJ, Stubbs LJ, Russell LB, Woychik RP (1994) A molecular model for the genetic and phenotypic characteristics of the mouse lethal yellow (Ay) mutation. Proceedings of the National Academy of Sciences of the United States of America 91:2562-6

Milatovich A, Hsieh CL, Bonaminio G, Tecott L, Julius D, Francke U (1992) Serotonin receptor 1c gene assigned to X chromosome in human (band q24) and mouse (bands D-F4). Human Molecular Genetics 1:681-4

Miller MW, Duhl DM, Vrieling H, Cordes SP, Ollmann MM, Winkes BM, Barsh GS (1993) Cloning of the mouse agouti gene predicts a secreted protein ubiquitously expressed in mice carrying the

lethal yellow mutation. Genes & Development 7:454-67

Mizuno TM, Mobbs CV (1999) Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. Endocrinology 140:814-7

Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S (1997) Congenital leptin defiency is associated with severe early-onset obesity in humans. Nature 387:903-908

Mountjoy KG, Mortrud MT, Low MJ, Simerly RB, Cone RD (1994) Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. Molecular Endocrinology 8:1298-308

Naggert JK, Fricker LD, Varlamov O, Nishina PM, Rouille Y, Steiner DF, Carroll RJ, Paigen BJ, Leiter EH (1995) Hyperproinsulinaemia in obese fat/fat mice associated with a carboxypeptidase E mutation which reduces enzyme activity. Nature Genetics 10:135-142

Nagle DL, McGrail SH, Vitale J, Woolf EA, Dussault BJ, Jr., DiRocco L, Holmgren L, Montagno J, Bork P, Huszar D, Fairchild-Huntress V, Ge P, Keilty J, Ebeling C, Baldini L, Gilchrist J, Burn P, Carlson GA, Moore KJ (1999) The mahogany protein is a receptor involved in suppression of obesity. Nature 398:148-52

Neel JV (1999) The "thrifty genotype" in 1998. Nutr. Rev. 57(5 Pt 2):S2-9

Nevanlinna HR (1972) The Finnish population structure. A genetic and genealogical study. Hereditas 71:195-236

Noben-Trauth K, Naggert JK, North MA, Nishina PM (1996) A candidate gene for the mouse mutation tubby. Nature 380:534-538

Nonogaki K, Strack AM, Dallman MF, Tecott LH (1998) Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT2C receptor gene. Nature Medicine 4:1152-6

Norio R (2000) Suomi-neidon geenit. Otava, Helsinki

Norman RA, Leibel RL, Chung WK, Power-Kehoe L, Chua SC, Jr., Knowler WC, Thompson DB, Bogardus C, Ravussin E (1996) Absence of linkage of obesity and energy metabolism to markers flanking homologues of rodent obesity genes in Pima Indians. Diabetes 45:1229-1232

Norman RA, Thompson DB, Foroud T, Garvey WT, Bennett PH, Bogardus C, Ravussin E (1997) Genomewide search for genes influencing percent body fat in Pima Indians: suggestive linkage at chromosome 11q21-q22. Pima Diabetes Gene Group. American Journal of Human Genetics 60:166-173

North MA, Naggert JK, Yan Y, Noben-Trauth K, Nishina PM (1997) Molecular characterization of TUB, TULP1, and TULP2, members of the novel tubby gene family and their possible relation to ocular diseases. Proceedings of the National Academy of Sciences of the United States of America 94:3128-33

O'Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. American Journal of Human Genetics 63:259-66

Ohlemiller KK, Hughes RM, Mosinger-Ogilvie J, Speck JD, Grosof DH, Silverman MS (1995) Cochlear and retinal degeneration in the tubby mouse. Neuroreport 6:845-9

Olefsky JM, Kolterman OG (1981) Mechanisms of insulin resistance in obesity and noninsulin-dependent (type II) diabetes. American Journal of Medicine 70:151-68

Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, Barsh GS (1997) Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein [published erratum appears in Science 1998 Sep 11;281(5383):1615]. Science 278:135-8

Olson JM, Wijsman EM (1993) Linkage between quantitative trait and marker loci: methods using all relative pairs. Genetic Epidemiology 10:87-102

O'Rahilly S, Gray H, Humphreys PJ, Krook A, Polonsky KS, White A, Gibson S, Taylor K, Carr C (1995)

Brief report: impaired processing of prohormones associated with abnormalities of glucose homeostasis and adrenal function. New England Journal of Medicine 333:1386-90

Ott J (1996) Complex traits on the map. Nature 379:772-3

Ott J (1995) How do you compute a lod score? Nature Genetics 11:354-5

Ott J (2000) Predicting the range of linkage disequilibrium. Proceedings of the National Academy of Sciences of the United States of America 97:2-3

Ozata M, Ozdemir IC, Licinio J (1999) Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects [published erratum appears in J Clin Endocrinol Metab 2000 Jan;85(1):416]. Journal of Clinical Endocrinology & Metabolism 84:3686-95

Pajukanta P, Cargill M, Viitanen L, Nuotio I, Kareinen A, Perola M, Terwilliger JD, Kempas E, Daly M, Lilja H, Rioux JD, Brettin T, Viikari JSA, Rönnemaa T, Laakso M, Lander ES, Peltonen L (2000) Two Loci on Chromosomes 2 and X for Premature Coronary Heart Disease Identified in Early- and Late-Settlement Populations of Finland. American Journal of Human Genetics 67:1481-1493

Pajukanta P, Nuotio I, Terwilliger JD, Porkka KV, Ylitalo K, Pihlajamaki J, Suomalainen AJ, Syvanen AC, Lehtimaki T, Viikari JS, Laakso M, Taskinen MR, Ehnholm C, Peltonen L (1998) Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. Nature Genetics 18:369-73

Pajukanta P, Terwilliger JD, Perola M, Hiekkalinna T, Nuotio I, Ellonen P, Parkkonen M, Hartiala J, Ylitalo K, Pihlajamaki J, Porkka K, Laakso M, Viikari J, Ehnholm C, Taskinen MR, Peltonen L (1999) Genomewide scan for familial combined hyperlipidemia genes in Finnish families, suggesting multiple susceptibility loci influencing triglyceride, cholesterol, and apolipoprotein B levels. American Journal of Human Genetics 64:1453-63

Palomaki H, Partinen M, Erkinjuntti T, Kaste M (1992) Snoring, sleep apnea syndrome, and stroke. Neurology 42:75-81; discussion 82

Panskepp J, Reilly P, Bishop P, Meeker RB, Vilberg TR, Kastin AJ (1976) Effects of alpha-MSH on motivation, vigilance and brain respiration. Pharmacology, Biochemistry & Behavior 5:59-64

Patten JL, Johns DR, Valle D, Eil C, Gruppuso PA, Steele G, Smallwood PM, Levine MA (1990) Mutation in the gene encoding the stimulatory G protein of adenylate cyclase in Albright's hereditary osteodystrophy [see comments]. New England Journal of Medicine 322:1412-9

Pekurinen M, Pokka-Vuento M, Salo H, Idänpään-Heikkilä U (2000) Lihavuus ja terveysmenot Suomessa 1997. Suomen Lääkärilehti 55:11-16

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F (1995) Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540-3

Peltonen L, Jalanko A, Varilo T (1999) Molecular genetics of the Finnish disease heritage. Human Molecular Genetics 8:1913-1923

Pericak-Vance MA, Bebout JL, Gaskell PC, Jr., Yamaoka LH, Hung WY, Alberts MJ, Walker AP, Bartlett RJ, Haynes CA, Welsh KA, et al. (1991) Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. American Journal of Human Genetics 48:1034-50

Perola M, Kainulainen K, Pajukanta P, Terwilliger JD, Hiekkalinna T, Ellonen P, Kaprio J, Koskenvuo M, Kontula K, Peltonen L (2000) Genome-wide scan of predisposing loci for increased diastolic blood pressure in Finnish siblings. Journal of Hypertension 18:1579-1585

Perone MJ, Ahmed I, Linton EA, Castro MG (1996) Procorticotrophin releasing hormone is endoproteolytically processed by the prohormone convertase PC2 but not by PC1 within stably transfected CHO-K1 cells. Biochemical Society Transactions 24:497S

Peroutka SJ, Schmidt AW, Sleight AJ, Harrington MA (1990) Serotonin receptor "families" in the central nervous system: an overview. Ann. N Y Acad. Sci. 600:104-112

Pietiläinen KH, Kaprio J, Rissanen A, Winter T, Rimpelä A, Viken RJ, Rose RJ (1999) Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: a study of 4884 twins and 2509 singletons. International Journal of Obesity & Related Metabolic Disorders 23:107-15

Pietinen P, Vartiainen E, Mannisto S (1996) Trends in body mass index and obesity among adults in Finland from 1972 to 1992. International Journal of Obesity & Related Metabolic Disorders 20:114-120

Pilia G, Hughes-Benzie RM, MacKenzie A, Baybayan P, Chen EY, Huber R, Neri G, Cao A, Forabosco A, Schlessinger D (1996) Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. Nature Genetics 12:241-7

Poggioli R, Vergoni AV, Bertolini A (1986) ACTH-(1-24) and alpha-MSH antagonize feeding behavior stimulated by kappa opiate agonists. Peptides 7:843-8

Post GB, Kemper HC (1993) Nutrient intake and biological maturation during adolescence. The Amsterdam growth and health longitudinal study. European Journal of Clinical Nutrition 47:400-8

Pratt SC, Daly MJ, Kruglyak L (2000) Exact multipoint quantitative-trait linkage analysis in pedigrees by variance components. American Journal of Human Genetics 66:1153-7

Preece MA (1996) The genetic contribution to stature. Hormone Research 45:56-8

Rahkonen O, Lundberg O, Lahelma E, Huuhka M (1998) Body mass and social class: a comparison of Finland and Sweden in the 1990s. Journal of Public Health Policy 19:88-105

Reed DR, Ding Y, Xu W, Cather C, Green ED, Price RA (1996) Extreme obesity may be linked to markers flanking the human OB gene. Diabetes 45:691-694

Rice T, Perusse L, Bouchard C, Rao DC (1996) Familial clustering of abdominal visceral fat and total fat mass: the Quebec Family Study. Obesity Research 4:253-61

Risch N (1990a) Linkage strategies for genetically complex traits. I. Multilocus models. American Journal of Human Genetics 46:222-8

Risch N (1990b) Linkage strategies for genetically complex traits. II. The power of affected relative pairs. American Journal of Human Genetics 46:229-41

Risch N, Zhang H (1995) Extreme discordant sib pairs for mapping quantitative trait loci in humans. Science 268:1584-9

Rissanen AM, Heliovaara M, Knekt P, Reunanen A, Aromaa A (1991) Determinants of weight gain and overweight in adult Finns. European Journal of Clinical Nutrition 45:419-30

Roberts DF, Billewicz WZ, McGregor IA (1978) Heritability of stature in a West African population. Annals of Human Genetics 42:15-24

Rosenbaum M, Leibel RL (1988) Pathophysiology of childhood obesity. Advances in Pediatrics 35:73-137

Rosenbaum M, Leibel RL, Hirsch J (1997) Obesity. New England Journal of Medicine 337:396-407

Rossi M, Kim MS, Morgan DG, Small CJ, Edwards CM, Sunter D, Abusnana S, Goldstone AP, Russell SH, Stanley SA, Smith DM, Yagaloff K, Ghatei MA, Bloom SR (1998) A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. Endocrinology 139:4428-31

Rotimi CN, Comuzzie AG, Lowe WL, Luke A, Blangero J, Cooper RS (1999) The quantitative trait locus on chromosome 2 for serum leptin levels is confirmed in African-Americans. Diabetes 48:643-4

Saltzman AG, Morse B, Whitman MM, Ivanshchenko Y, Jaye M, Felder S (1991) Cloning of the human serotonin 5-HT2 and 5-HT1C receptor subtypes. Biochemical & Biophysical Research Communications 181:1469-78

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. Cold Spring Harbor Laboratory Press, New York, New York

Sanchis D, Fleury C, Chomiki N, Goubern M, Huang Q, Neverova M, Gregoire F, Easlick J, Raimbault S,

Levi-Meyrueis C, Miroux B, Collins S, Seldin M, Richard D, Warden C, Bouillaud F, Ricquier D (1998) BMCP1, a novel mitochondrial carrier with high expression in the central nervous system of humans and rodents, and respiration uncoupling activity in recombinant yeast. Journal of Biological Chemistry 273:34611-5

Santer R, Schneppenheim R, Dombrowski A, Gotze H, Steinmann B, Schaub J (1997) Mutations in GLUT2, the gene for the liver-type glucose transporter, in patients with Fanconi-Bickel syndrome [published erratum appears in Nat Genet 1998 Mar;18(3):298]. Nature Genetics 17:324-6

Sargent PA, Sharpley AL, Williams C, Goodall EM, Cowen PJ (1997) 5-HT2C receptor activation decreases appetite and body weight in obese subjects. Psychopharmacology 133:309-12

Schork NJ (1993) Extended multipoint identity-by-descent analysis of human quantitative traits: efficiency, power, and modeling considerations. American Journal of Human Genetics 53:1306-19

Schwindinger WF, Miric A, Zimmerman D, Levine MA (1994) A novel Gs alpha mutant in a patient with Albright hereditary osteodystrophy uncouples cell surface receptors from adenylyl cyclase. Journal of Biological Chemistry 269:25387-91

Seidell JC (1995b) The impact of obesity on health status: some implications for health care costs. International Journal of Obesity & Related Metabolic Disorders 19:S13-6

Seidell JC (1995a) Obesity in Europe. Obesity Research 3:89s-93s

Shiang R, Thompson LM, Zhu YZ, Church DM, Fielder TJ, Bocian M, Winokur ST, Wasmuth JJ (1994) Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. Cell 78:335-42

Shih JC, Zhu Q, Chen K (1996) Determination of transcription initiation sites and promoter activity of the human 5-HT2A receptor gene. Behavioural Brain Research 73:59-62

Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL (1997) Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. Genes & Development 11:593-602

Silventoinen K (2000) Body-height: Determinants and associations with social position and adult health. (Doctoral Thesis). In: Department of Public Health UoH (ed) Kansanterveystieteen julkaisuja M174/2000, Helsinki

Silventoinen K, Kaprio J, Lahelma E, Koskenvuo M (2000) Relative effect of genetic and environmental factors on body height: differences across birth cohorts among Finnish men and women. American Journal of Public Health 90:627-30

Silvers WK, Russel ES (1979) The Coat Colors of Mice: a Model for Mammalian Gene Action and Interaction. Springler-Verlag, New York

Simansky KJ (1996) Serotonergic control of the organization of feeding and satiety. Behavioural Brain Research 73:37-42

Sina M, Hinney A, Ziegler A, Neupert T, Mayer H, Siegfried W, Blum WF, Remschmidt H, Hebebrand J (1999) Phenotypes in three pedigrees with autosomal dominant obesity caused by haploinsufficiency mutations in the melanocortin-4 receptor gene. American Journal of Human Genetics 65:1501-7

Solomon PJ, Thompson EA, Rissanen A (1983) The inheritance of height in a Finnish population. Annals of Human Biology 10:247-56

Spanswick D, Smith MA, Groppi VE, Logan SD, Ashford ML (1997) Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. Nature 390:521-5

Spielman RS, McGinnis RE, Ewens WJ (1994) The transmission/disequilibrium test detects cosegregation and linkage. American Journal of Human Genetics 54:559-60; discussion 560-3

Stirling B, Cox NJ, Bell GI, Hanis CL, Spielman RS, Concannon P (1995) Identification of microsatellite markers near the human ob gene and linkage studies in NIDDM-affected sib pairs. Diabetes

44:999-1001

Strain GW, Zumoff B (1992) The relationship of weight-height indices of obesity to body fat content. Journal of the American College of Nutrition 11:715-8

Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD (1998) A Leptin Missense Mutation Associated With Hypogonadism and Morbid Obesity. Nature Genetics 18:213-215

Stubdal H, Lynch CA, Moriarty A, Fang Q, Chickering T, Deeds JD, Fairchild-Huntress V, Charlat O, Dunmore JH, Kleyn P, Huszar D, Kapeller R (2000) Targeted deletion of the tub mouse obesity gene reveals that tubby is a loss-of-function mutation. Molecular & Cellular Biology 20:878-82

Stunkard AJ, Foch TT, Hrubec Z (1986a) A twin study of human obesity. Journal of American Medical Association 256:51-54

Stunkard AJ, Harris JR, Pedersen NL, McClearn GE (1990) The body-mass index of twins who have been reared apart. New England Journal of Medicine 322:1483-1487

Stunkard AJ, Sorensen TI, Hanis C, Teasdale TW, Chakraborty R, Schull WJ, Schulsinger F (1986b) An adoption study of human obesity. New England Journal of Medicine 314:193-8

Suarez BK, Rice J, Reich T (1978) The generalized sib pair IBD distribution: its use in the detection of linkage. Annals of Human Genetics 42:87-94

Superti-Furga A, Eich G, Bucher HU, Wisser J, Giedion A, Gitzelmann R, Steinmann B (1995) A glycine 375-to-cysteine substitution in the transmembrane domain of the fibroblast growth factor receptor-3 in a newborn with achondroplasia. European Journal of Pediatrics 154:215-9

Taillon-Miller P, Bauer-Sardina I, Saccone NL, Putzel J, Laitinen T, Cao A, Kere J, Pilia G, Rice JP, Kwok PY (2000) Juxtaposed regions of extensive and minimal linkage disequilibrium in human Xq25 and Xq28. Nature Genetics 25:324-8

Tanner JM (1992) Growth as a measure of the nutritional and hygienic status of a population. Hormone Research 38:106-15

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir G, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA, Tepper RI (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263-1271

Taylor BA, Phillips SJ (1996) Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics 34:389-98

Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D (1995) Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. Nature 374:542-6

Terwilliger JD (1995) A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. American Journal of Human Genetics 56:777-87

Terwilliger JD, Göring HHH (2000a) Gene mapping in the 20th and 21st centuries: Statistical methods, data analysis, and experimental design. Human Biology :63-132

Terwilliger JD, Ott J (1992) A haplotype-based 'haplotype relative risk' approach to detecting allelic associations. Human Heredity 42:337-46

Terwilliger JD, Shannon WD, Lathrop GM, Nolan JP, Goldin LR, Chase GA, Weeks DE (1997) True and false positive peaks in genomewide scans: applications of length-biased sampling to linkage mapping. American Journal of Human Genetics 61:430-8

Terwilliger JD, Zollner S, Laan M, Paabo S (1998) Mapping genes through the use of linkage disequilibrium generated by genetic drift: 'drift mapping' in small populations with no demographic expansion. Human Heredity 48:138-54

Thompson DB, Ravussin E, Bennett PH, Bogardus C (1997) Structure and sequence variation at the human leptin receptor gene in lean and obese Pima Indians. Human Molecular Genetics 6:675-679

Thornton JE, Cheung CC, Clifton DK, Steiner RA (1997) Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. Endocrinology 138:5063-6

Troiano RP, Frongillo EA, Jr., Sobal J, Levitsky DA (1996) The relationship between body weight and mortality: a quantitative analysis of combined information from existing studies. International Journal of Obesity & Related Metabolic Disorders 20:63-75

Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P (2000) Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. Journal of Clinical Investigation 106:253-262

Vaisse C, Clement K, Guy-Grand B, Froguel P (1998) A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nature Genetics 20:113-4

Walder K, Hanson RL, Kobes S, Knowler WC, Ravussin E (2000) An autosomal genomic scan for loci linked to plasma leptin concentration in Pima Indians. International Journal of Obesity & Related Metabolic Disorders 24:559-65

Valverde P, Healy E, Jackson I, Rees JL, Thody AJ (1995) Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nature Genetics 11:328-30

Vartiainen E, Puska P, Jousilahti P, Korhonen HJ, Tuomilehto J, Nissinen A (1994) Twenty-year trends in coronary risk factors in north Karelia and in other areas of Finland. International Journal of Epidemiology 23:495-504

Weeks DE, Lange K (1992) A multilocus extension of the affected-pedigree-member method of linkage analysis. American Journal of Human Genetics 50:859-68

Weiss KM, Terwilliger JD (2000) How many diseases does it take to map a gene with SNPs? Nature Genetics 26:151-157

Venter JC et al. (2001) The sequence of the human genome. Science 291:1304-1351

Wertheimer E, Litvin Y, Ebstein RP, Bennet ER, Barbetti F, Accili D, Taylor SI (1994) Deletion of exon 3 of the insulin receptor gene in a kindred with a familial form of insulin resistance. Journal of Clinical Endocrinology & Metabolism 78:1153-8

Wessman M, Kallela M, Hovatta I, Marttila P, Kaunisto M, Koskenvuo M, Honkasalo ML, Palotie A (1998) A genome-wide search for genes predisposing to migraine in Finnish twins (abstract). American Journal of Human Genetics 63 (suppl):A314

WHO (1997) Obesity: preventing and managing the global epidemic. Report of a WHO Consultation on Obesity, Geneva 3-5, June 1997. World Health Organization Techinical Report Series. Vol. 210. World Health Organization, Geneva

WHO (1995) Physical Status: The Use and Interpretation of Anthropometry: WHO Technical Reports Series no. 854. World Health Organization Expert Committee, Geneva

Wikberg JE (1999) Melanocortin receptors: perspectives for novel drugs. European Journal of Pharmacology 375:295-310

Williams JT, Blangero J (1999) Comparison of variance components and sibpair-based approaches to quantitative trait linkage analysis in unselected samples. Genetic Epidemiology 16:113-34

Williams JT, Duggirala R, Blangero J (1997) Statistical properties of a variance components method for quantitative trait linkage analysis in nuclear families and extended pedigrees. Genetic Epidemiology 14:1065-70

Williamson DF, Madans J, Anda RF, Kleinman JC, Kahn HS, Byers T (1993) Recreational physical activity and ten-year weight change in a US national cohort. International Journal of Obesity & Related Metabolic Disorders 17:279-86

Wilson GT, Nonas CA, Rosenblum GD (1993) Assessment of binge eating in obese patients. International Journal of Eating Disorders 13:25-33

Witte JS, Elston RC, Schork NJ (1996) Genetic dissection of complex traits. Nature Genetics 12:355-6;

discussion 357-8

Wolfe BE, Metzger E, Jimerson DC (1997) Research update on serotonin function in bulimia nervosa and anorexia nervosa. Psychopharmacology Bulletin 33:345-54

Xie E, Zhu L, Zhao L, Chang LS (1996) The human serotonin 5-HT2C receptor: complete cDNA, genomic structure, and alternatively spliced variant. Genomics 35:551-61

Yang YK, Ollmann MM, Wilson BD, Dickinson C, Yamada T, Barsh GS, Gantz I (1997) Effects of recombinant agouti-signaling protein on melanocortin action. Molecular Endocrinology 11:274-80

Yaswen L, Diehl N, Brennan MB, Hochgeschwender U (1999) Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. Nature Medicine 5:1066-70

Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S (1998) A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nature Genetics 20:111-2

Yeo GSH, Farooqi IS, Challis BG, Jackson RS, O'Rahilly S (2000) The role of melanocortin signalling in the control of body weight: evidence from human and murine genetic models. Qjm Monthly Journal of the Association of Physicians 93:7-14

Young TL, Penney L, Woods MO, Parfrey PS, Green JS, Hefferton D, Davidson WS (1999) A fifth locus for Bardet-Biedl syndrome maps to chromosome 2q31. American Journal of Human Genetics 64:900-4

Yuan X, Yamada K, Ishiyama-Shigemoto S, Koyama W, Nonaka K (2000) Identification of polymorphic loci in the promoter region of the serotonin 5-HT2C receptor gene and their association with obesity and type II diabetes. Diabetologia 43:373-6

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432

Zhou A, Bloomquist BT, Mains RE (1993) The prohormone convertases PC1 and PC2 mediate distinct endoproteolytic cleavages in a strict temporal order during proopiomelanocortin biosynthetic processing. Journal of Biological Chemistry 268:1763-9

Zhuchenko O, Wehnert M, Bailey J, Sun ZS, Lee CC (1996) Isolation, mapping, and genomic structure of an X-linked gene for a subunit of human mitochondrial complex I. Genomics 37:281-8